M11

09/176395 PCT/AU99/00705

AUSTRALIA

AU99/705

4

Patent Office
Canberra

I, LEANNE MYNOTT, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PP 5572 for a patent by THE UNIVERSITY OF QUEENSLAND filed on 31 August 1998.

COMMONWEALTH OF AUSTRALIA
AUSTRALIA
PATENT OFFICE

WITNESS my hand this
Sixth day of October 1999

L. Mynott

LEANNE MYNOTT
TEAM LEADER EXAMINATION
SUPPORT AND SALES

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

THIS PAGE BLANK (USPTO)

Regulation 3.2

The University of Queensland

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"A novel plant promoter and uses therefor"

The invention is described in the following statement:

Plants are subject to a variety of environmental and mechanical stimuli including stress. Although mechanical stress has been postulated to involve ethylene-mediated meristem morphogenesis (Selker *et al,* 1992), little is known about how mechanical stress induces ethylene production or the signal transduction process involved.

In work leading to the present invention, the inventors sought to identify and isolate promoters involved in mechanical stress-induced expression of genetic traits in *Vigna radiata* (mung bean). Mung bean plants are a useful model for physical and chemical induction of phenotypic expression of genetic traits due to their morphology, rapid growth rate and the ability to obtain a large number of uniform plants and, therefore, sufficient amounts of tissues to conduct analyses.

In accordance with the present invention, the inventors have isolated a promoter capable of induction following physical stimulus in cells in which the promoter is indigenous, i.e. cells of mung bean plants. The promoter is also capable of being induced by a range of chemical and other environmental stimuli. However, in cells in which the promoter is non-indigenous, the promoter is constitutively expressed. The promoter of the present invention is useful in the genetic manipulation of plants.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

One aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein, in its native form, the promoter is inducible in response to physical stimulation.

Another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein said promoter, in its native form, directs expression of a gene associated with ethylene production and is inducible by physical stimulation.

Yet another aspect of the present invention relates to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase and is inducible by physical stimulation.

Still another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase or another gene associated with ethylene biosynthesis and is inducible by physical stimulation.

Still yet another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding an ACC synthase having an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least 60% similarity to SEQ ID NO:2.

A further aspect of the present invention relates to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase and wherein said gene comprises a nucleotide sequence substantially as set forth in SEQ ID NO:1 or a sequence having at least 50% similarity thereto and/or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C.

Still another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, comprises a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:3 under low stringency conditions at 42°C.

Another aspect of the present invention provides a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs expression of a gene associated with ethylene biosynthesis and is inducible by physical stimulation.

Yet another aspect of the present invention is directed to a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs synthesis of an ACC synthase having an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least 60% similarity thereto.

Still yet another aspect of the present invention is directed to a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, it is native form, directs synthesis of an ACC synthase encoded by a gene comprising a nucleotide sequence substantially as set forth in SEQ ID NO:1 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C.

In still yet another aspect of the present invention, there is provided a modular promoter comprising a portion which is derived from a promoter which comprises, in its native form, a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:3 under low stringency conditions at 42°C.

Another aspect of the present invention contemplates a genetic construct comprising a promoter

or modular promoter each as herein defined or a derivative or homologue thereof, means to facilitate insertion of a nucleotide sequence downstream of and operably linked to said promoter and optionally a gene encoding a selectable marker.

A further aspect of the present invention provides a genetic construct comprising a promoter or modular promoter each as herein defined or a derivative or homologue thereof, a nucleotide sequence operably linked to said promoter and optionally a gene encoding a selectable marker.

Another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof which is capable of constitutive expression in cells in which the promoter is non-indigenous.

Yet another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein said promoter, in its native form, directs expression in response to physical stimulation of a gene associated with ethylene production and which promoter in a non-native host cell is constitutively expressed.

Still yet another aspect of the present invention relates to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression in response to physical stimulation of a gene encoding ACC synthase and which promoter in a non-native host cell is constitutively expressed.

A further aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase or another gene associated with ethylene biosynthesis and in a cell in which the promoter is indigenous, the promoter is inducible by physical stimulation whereas in a cell in which the promoter is non-indigenous, the promoter is constitutively

expressed.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a representation of the oligonucleotide primers used in Long Distance Inverse PCR.

Figure 2 is a diagrammatic representation showing generation of *Spe*I and *Xba*-I fragments of *AIM-1* promoter.

Figure 3 is a diagrammatic representation of the *AIM-1* promoter sequencing strategy.

Figure 4 is a representation of the nucleotide sequence of the *AIM-1* (2470 bp) promoter.

Figure 5 is a diagrammatic representation of the construction of a full length *AIM-1* promoter.

Figure 6A(i) - 6A(xii) are diagrammatic representations of plasmids pPZP2.5GuNt, pPZP2.5LuNt, pPZP1.4GuNt, pPZP1.4LuNt, pPZP35SGuNt, pPZP35SLuNt, pP2P017GuNt, pPZP023GuNt, pPZP07GuNt, pPZP045GuNt, pPZP088GuNt and pPZP1.1GuNt, respectively.

Figure 6B is a diagrammatic representation of the backbone vector pPZP111.

Figure 6C is a diagrammatic representation of the vector pGuNt.

Figure 7 are photographic representations showing (A) and (B) transgenic tobacco lines containing *AIM-1*:GUS gene assayed to visualise GUS activity; and (C) non-stained controls.

Figures 8(A) and (B) are graphical representations showing GUS activity in young tobacco plants, transformed with *AIM-1*:GUS and CaMV35S:GUS constructs. 2.5G#3-4 and 2.5G#7-3 are two independent transgenic lines containing full length *AIM-1* promoter fused to the GUS gene; 35SG#5-2 is a transgenic line containing CaMV35S promoter fused to the GUS gene. (A) is GUS activity per/mg protein. (B) is GUS activity per/g of fresh weight. MU is equal to 4-

methyl-umbrelliferone.

Figure 9 is a photographic representation of Southern analysis of three T2 homozygous independent tobacco transgenic lines (3-4, 7-3 and 10-3) containing the *AIM-1* promoter fused to the GUS gene, and one T2 homozygous tobacco transgenic line (5-2) containing the CaMV 35S promoter fused to the GUS gene. Genomic DNA was digested with *Eco*RI (E) or *Bam*HI (B) restriction enzymes. A ^{32}P-labelled DNA fragment containing the full GUS gene and Nos terminator was used as a probe. Lane 1 contained size markers. Lanes 2 and 3: line 3-4; lanes 4 and 5: line 7-3; lanes 6 and 7: line 10-3; lane 8: line 5-2.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification of a promoter directing expression of a gene. The gene encodes 1-aminocyclopropane-1-carboxylic acid synthase ("ACC synthase") and is inducible, in its native form, by physical stimuli (Botella *et al,* 1992; Botella *et al,* 1995). Referenced herein to "native form" with respect to a promoter means the promoter in cells in which the promoter is normally resident, i.e. indigenous. In the present case, cells for mung bean plants are cells in which the promoter is indigenous. Transfer of the promoter by genetic means to other plant cells is an example of cells carrying a non-indigenous promoter.

Accordingly, the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein, in its native form, the promoter is inducible in response to physical stimulation.

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein said promoter, in its native form, directs expression of a gene associated with ethylene production and inducible by physical stimulation.

Even more particularly, the present invention relates to an isolated nucleic acid molecule

comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase and inducible by physical stimulation.

In a related embodiment, the present invention relates to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase or another gene associated with ethylene biosynthesis and is inducible by physical stimulation.

Although the present invention is exemplified by the identification and isolation of the promoter directing synthesis of ACC synthase from *Vigna radiata* (mung bean), the present invention extends to any promoter which, in its native form, is inducible in response to physical stimulation and which comprises a nucleotide sequence having at least about 50% similarity to the nucleotide sequence set forth in SEQ ID NO:1 and/or nucleotide sequence capable of hybridizing to the nucleotide sequence of SEQ ID NO:1 under low stringency conditions at 42°C.

Examples of promoters contemplated by the present invention include but are not limited to promoters directing expression of genes associated with ethylene biosynthesis such as the gene encoding ACC synthase.

The gene encoding ACC synthase from mung bean is referred to as *"AIM-1"*. ACC synthase from mung bean comprises the amino acid sequence substantially as set forth in SEQ ID NO:2.

Accordingly, another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding an ACC synthase having an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least 60% similarity to SEQ ID NO:2.

The percentage similarity at the amino acid or nucleotide sequence level is generally to a portion comprising at least about 20 contiguous amino acids or at least about 60 contiguous nucleotide bases. Preferably, however, the comparison is made to the entire amino acid sequence or entire nucleotide sequence. Alternative percentage similarities include at least about 70%, at least about 80%, at least about 90% and at least about 95% or above or discrete percentages there between.

Genes encoding ACC synthase enzymes not having 100% similarity to SEQ ID NO:2 include derivatives and homologous of the mung bean enzyme. A derivative includes parts, fragments, mutants and fusions of the mung bean ACC synthase defined in SEQ ID NO:2 including ACC synthase enzymes having one or more amino acid substitutions, additions and/or deletions to the amino acid sequence of SEQ ID NO:2. Homologues include enzymes from closely or distantly related plants including fungi.

A particularly preferred promoter of the present invention directs expression of *AIM-1*. The nucleotide sequence of *AIM-1* is set forth in SEQ ID NO:1.

According to this embodiment, there is provided an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter in its native form directs expression of a gene encoding ACC synthase and wherein said gene comprises a nucleotide sequence substantially as set forth in SEQ ID NO:1 or a sequence having at least 50% similarity thereto and/or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M

to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

Alternative percentage similarities include those set forth above.

Nucleotide sequences not having 100% similarity to SEQ ID NO:1 include derivatives and homologues of mung bean *AIM-1*. A derivative includes, parts, fragments, mutants and fusions of the mung bean *AIM-1* defined in SEQ ID NO:1 including *AIM-1* genes having one or more nucleotide substitutions, additions and/or deletions to the nucleotide sequence of SEQ ID NO:1. Homologues include genes from closely or distantly related plants including fungi.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. The terms "homology" and "identity" may be used to substitute for "similarity".

Most preferably, the promoter of the present invention comprises a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a functional derivative or homologue thereof.

Accordingly, another aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter in its native form comprises a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:3 under low stringency conditions at 42°C.

The promoter of the present invention is useful in the development of genetic constructs to express heterologous nucleotide sequences placed downstream of, and operably linked to, the promoter.

The term "promoter" is used herein in its most general sense and refers to any nucleotide sequence which binds RNA polymerase and directs same to a transcriptional start site whereupon a gene or other nucleotide sequence downstream of said promoter is transcribed. A nucleotide sequence "downstream" of the promoter is also said to be "relative" to the promoter or "operably" linked to the promoter.

The term "genetic construct" is used in its broadest sense to include an isolated nucleic acid molecule comprising a sequence of nucleotides.

The genetic construct is conveniently engineered so as to include means to facilitate insertion of a nucleotide sequence in a region 3′ of the promoter to place the nucleotide sequence downstream of and operably linked to the promoter for their transcription. Such a "means" includes but is not limited to a restriction endonuclease-mediated insertion, homologous recombination, transposon insertion, PCR mediated insertion and random insertion. Preferably, the means is a restriction endonuclease site. Generally, the inserted restriction site is unique to the genetic construct or may be represented, for example, twice but separated by a nucleic acid sequence which is deleted upon restriction digestion of the genetic construct. The required nucleotide sequence to be transcribed is then inserted into the deleted region.

The genetic construct of the present invention may comprise solely the promoter and optionally a nucleotide sequence downstream thereof or, alternatively, may comprise additional nucleotide sequences constituting promoter regulatory region(s), transcribed sequence regulatory regions, a marker (eg. antibiotic resistance, chemical compound resistance or enzyme), autonomous replication region and/or genome integration sequence. The promoter may be the naturally occurring promoter or may be an active fragment or part thereof or a derivative, analogue or homologue of the promoter.

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a promoter or modular promoter each as herein defined or a derivative or homologue thereof, means to facilitate insertion of a nucleotide sequence operably linked to said promoter and optionally a gene encoding a selectable marker.

More particularly, this aspect provides a genetic construct comprising a promoter or modular promoter as herein defined or a derivative or homologue thereof, one or more unique restriction sites down stream of said promoter to enable the insertion of a heterologous nucleotide sequence operably linked to said promoter and a gene encoding a selectable marker.

In a related embodiment, the present invention provides a genetic construct comprising a promoter or modular promoter each as herein defined or a derivative or homologue thereof, a nucleotide sequence operably linked to said promoter and optionally a gene encoding a selectable marker.

The promoter of the present invention, in its native form (i.e. in cells in which it is indigenous), is inducible by physical stimulus which includes mechanical stress, movement, vibration, air pressure, water stress and the like. Other non-mechanical stimuli also induce the instant promoter including auxins, abscisic acid, salt concentration amongst others. Non-mechanical stimuli include environmental stimuli such as but not limited to chemical induction of the promoter. The promoter may also be developmentally regulated and/or may be tissue or organ specific.

The identification of a promoter capable of induction by physical or mechanical stimuli provides a particularly useful basis for developing a range of genetically altered plants. For example, air movement may be used to activate expression of a nucleotide sequence operably linked to the subject promoter. This may be useful during the commercial cultivation of large numbers of plants. Generating air movement such as generated by fanning, or a change in air pressure over and/or around the plants can be used to activate expression of the promoter. Alternatively, or in addition, water droplets generated mechanically or by controlling humidity may be used to stimulate promoter activity. Heterologous nucleotide sequences operably linked to the promoter

are then expressed. Such heterologous sequences may encode, for example, resistance to insect or other pathogens, salt tolerance, enzymes which manipulate the flow of metabolites down particular biochemical pathways, enzymes which alter the nutritional content of certain types of plant tissues including seeds and other reproductive parts and antisense, co-suppression, ribozyme or deoxyribozyme molecules to down regulate expression of an endogenous gene. Examples of the latter would be to render a plant male or female sterile, to alter biochemical pathways or to otherwise alter the characteristics of the target plant, such as to inhibit ethylene biosynthesis or to delay synthesence.

Accordingly, another aspect of the present invention contemplates a method of altering a characteristic of a plant said method comprising introducing a genetic construct into a cell or group of cells of a plant, said genetic construct comprising a promoter as herein defined and a nucleotide sequence operably linked to said promoter and wherein said nucleotide sequence facilitates the altering of said plant characteristic, regenerating a plant or plantlet from said cell or group of cells carrying said genetic construct and growing or subjecting said plant or plantlet to conditions sufficient to induce the promoter in said genetic construct.

The genetically altered plant may be subjected to physical stimulus such as mechanical stress in order to induce the promoter. Alternative forms of stimulus, however, are also contemplated by the subject invention such as water droplets, air movement, air pressure and chemical stimuli such as auxins. The promoter may also be constitutively expressed.

An altered characteristic may be readily determined by comparing a transgenic plant with a non-transgenic plant of the same species. The comparison may be at the biochemical, physiological or visual level. Altered characteristics include but are not limited to resistance to plant viruses, bacteria, fungi, nematodes and other pathogens, improved nutritional value (eg. using sunflower high sulphur gene), an expression of an "antibody" (often referred to as a "plantabody"), altered biochemical pathways, altered fertility, altered flower colour amongst many other characteristics.

The promoter of the present invention is inducible by a range of stimuli including physical, environmental, chemical and genetic. The promoter comprises, therefore, different regulatory

areas for different stimuli. The present invention contemplates the manipulation of the subject promoter such that it is inducible by a particular stimulus or stimuli.

Accordingly, another aspect of the present invention provides a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs expression of a gene associated with ethylene biosynthesis and is inducible by physical stimulation.

More particularly, the present invention is directed to a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, in its native form, directs synthesis of an ACC synthase having an amino acid sequence substantially as set forth in SEQ ID NO:2 or an amino acid sequence having at least 60% similarity thereto.

Even more particularly, the present invention is directed to a modular promoter, said modular promoter comprising at least one portion which is derived from a promoter which, it is native form, directs synthesis of an ACC synthase encoded by a gene comprising a nucleotide sequence substantially as set forth in SEQ ID NO:1 or a nucleotide sequence having at least 50% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:1 under low stringency conditions at 42°C.

Still more particularly, the present invention provides a modular promoter comprising a portion which is derived from a promoter which comprises, in its native form, a nucleotide sequence substantially as set forth in SEQ ID NO:3 or a nucleotide sequence having at least 25% similarity thereto or a nucleotide sequence capable of hybridizing to SEQ ID NO:3 under low stringency conditions at 42°C.

A "modular" promoter is considered as an example of a "derivative". Another derivative contemplated by the present invention includes the deletion of negatively acting *cis* element(s). This aspect of the present invention is predicated on the observation of high expression of the promoter in the presence of the protein synthesis inhibitor, cycloheximide, which inhibits production of a highly unstable, short-lived negative regulator (transcription factor) of the

subject promoter. Accordingly, by deleting the negative *cis* element(s), higher inducible or even constitutive expression of the promoter may be obtained.

Another aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof which is capable of constitutive expression in cells in which the promoter is non-indigenous.

This aspect of the present invention is predicated on the surprising observation that the promoter of the present invention, when placed in plant cells in which it is not indigenous, i.e. non-mung bean cells, is constitutively expressed. Although no intending to limit the present invention to any one theory or mode of action, it is proposed that in cells in which the promoter is indigenous, a negative regulatory molecule prevents constitutive expression of the promoter. This negative regulatory molecule would not normally be present in other plant cells and, hence, the promoter is constitutively expressed.

Accordingly, another aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof, wherein said promoter, in its native form, directs expression in response to physical stimulation of a gene associated with ethylene production and in which in a non-native host cell is constitutively expressed.

More particularly, a further aspect of the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary sequence of nucleotides defining a promoter or a derivative or homologue thereof wherein said promoter, in its native form, directs expression of a gene encoding ACC synthase or another gene associated with ethylene biosynthesis and in a cell in which the promoter is indigenous, the promoter is inducible by physical stimulation whereas in a cell in which the promoter is non-indigenous, the promoter is constitutively expressed.

The present invention further contemplates a transgenic plant carrying the promoter of the present invention or parts, limbs, flowers, petals, reproductive portions or seeds thereof or progeny or clone thereof.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Detection of mechanical strain-induced gene

A gene encoding 1-aminocyclopropane-1-carboxylic acid synthase ("ACC Synthase"), induced *inter alia* by mechanical strain, auxin and salt stress was isolated according to Botella *et al* (1992;1995). The cDNA sequence and corresponding amino acid sequence is shown in SEQ ID NO:1. The amino acid sequence alone is shown in SEQ ID NO:2.

EXAMPLE 2

Cloning of the ACC Synthase gene (*AIM-1*) promoter

(a) Recirculation of DNA

Ten micrograms of genomic DNA isolated by CsCl purification was digested with 2.5U/μg of *Hind*III in the presence of 0.1M spermidine, extracted with 1 volume phenol:chloroform:isamyl alcohol (25:24:1) and precipitated by addition of 0.1 vol NaOAc and 2 volumes EtOH. DNA was then re-ligated with 9 Weiss units of T4 DNA ligase and purified using Bresatec's Bresa Clean Kit. The effectiveness of recircularisation was analyzed by gel electrophoresis.

(b) Long Distance Inverse Polymerase Chain Reaction (LDIPCR) procedure

A reaction mixture of 2mM $MgSO_4$ pH 9.1, containing 60mM Tris-SO4, 18mM (NH_4) $2SO_4$, 0.2mM of each dNTP, 0.2μM of NSE oligonucleotide primers (see Figure 1), sterile water and 300ng of recircularised genomic DNA was prepared in a total volume of 40μl. The reaction mixture was vortexed and briefly spun prior to incubation at 94°C to prevent non-specific primer interactions. Before initialising the thermal cycle, 10μl of sterile water

containing 1μL of Life Technologies' eLONGase enzyme mix (Taql/Vent polymerases) was added to the reaction and mixed by pipetting. An equal volume of mineral oil was layered over the mix to prevent evaporation. The optimised PCR parameters are shown in Table 1.

TABLE 1

PCR profile times and temperatures used during amplification and reamplification protocols.

Optimised Temperatures and Times				
Amplification	Initial Step	Denaturation	Anneal and Extension	
	60 sec. 94°C	30 sec. 94°C	480 sec. 68°C	
		45 cycles		
Reamplification	Initial Step	Denaturation	Anneal	Extension
	60 sec. 94°C	30 sec. 94°C	30 sec. 62°C	480 sec. 68°C
		35 cycles		

After the final step of thermal cycling, 1 volume of chloroform-isoamyl alcohol (24:1) was added to remove the oil layer and the samples were stored at 4°C.

Cloning Strategy

The circularised genomic DNA was first amplified with oligonucleotide primers NSE-1 and NSE-2 (refer to Figure 1). The products of this first amplification were further reamplified using either NSE-3/NSE-4 or NSE-5/NSE-6 (Figure 1). Electrophoretic analysis of the amplification products, generated with both combinations of primers, revealed a DNA fragment of approximately 4kb.

EXAMPLE 3

Analysis of 4kb fragment

The 4kb product obtained with NSE3/NSE-4 (Example 2) was excised from the gel and purified with glassmilk (Bresatec's Bresa Clean). As attempts at cloning the 4kb product were initially unsuccessful, alternative strategies were devised. The purified 4kb product was digested with *Xba*I and two fragments of 1.3 kb and 0.9 kb were sub-cloned into the vector pGEM11 (Promega corporation, USA), which had been previously digested with *Xba*I giving the plasmids pGX1.3 and pGX0.9, respectively. The 4kb fragment was also digested with *Spe*I and blunt-ended before cloning the digestion products into pGEM11 (previously digested with *Xho*I and blunt-ended). As a result, two *Spe*I fragments of 1.1kb and 1.4kb were sub-cloned and the plasmids named pGS1.1 and pGS1.4, respectively. The 1.4kb fragment did not show any *Spe*I recognition sequences in one of its ends, indicating that some exonuclease activity had taken place during the blunt-ending process. This is summarised in Figure 2.

EXAMPLE 4

Reconstruction and sequencing of the 2.5kb *AIM-1* promoter region

The sequencing strategy for the *AIM-1* promoter is shown in Figure 3. Sequencing was performed using the dideoxy chain termination method (Sanger *et al,* 1977) using a Applied Biosystems kit (Applied Biosystems, USA). Analysis of the sequences revealed that the four clones partially overlapped. The 1.3 *Xba*I and 1.1kb *Spe*I fragments contained the 5' untranslated region of the *AIM-1* cDNA, confirming that this region is upstream of the *AIM-1* gene. As a result, a partial restriction map for a 2.5kb region of the 4kb DNA fragment was generated. The nucleotide sequence of the *AIM-1* promoter is shown in Figure 4 and in SEQ ID NO:3.

With this information in hand, the promoter region was reconstructed by the following strategy. pGS1.4 was digested with *Hind*III and blunt ended. The promoter insert was then

excised with *Spe*I, obtaining a 1.4kb fragment with blunt-*Spe*I ends.

pGS1.1 was linearised with *Sal*I and blunt ended. Later the linearised construct was digested with *Spe*I resulting in a linearised vector with blunt-*Spe*I ends containing the 3' end of the promoter region. The fragment excised in (a) was ligated into (b) to reconstruct the 2.5kb *AIM-1* promoter. Figure 5 is a diagrammatic representation of the construction of the full length *AIM-1* promoter.

EXAMPLE 5

Characterization of *AIM-1* promoter

(a) Generation of deletion fragments and chimeric gene constructs

To fully characterise the *AIM-1* promoter, two different lengths of the promoter sequence were used: the entire 2.5kb sequence and a 1.4kb fragment upstream of the first ATG codon. β-Glucuronidase (GUS) and luciferase (*LUC*) reporter genes were each ligated to one or other of the promoter fragments and to the 3' terminator region from the *Agrobacterium tumefaciens* nopaline synthase gene (NOS) to generate a series of chimeric gene constructs.

A series of 7 deletions in the promoter region were also generated, starting from 170 base-pairs upstream of the first ATG codon. Each of these was likewise ligated to the NOS 3' terminator region and to the GUS reporter gene. Intermediate vectors containing each of the promoter fragments (0.17, 0.23, 0.45, 0.70, 0.88, 1.1, 1.4, 1.6 or 2.5kb) ligated to the GUS or *LUC* reporter genes and NOST were generated in pBluescript. For control purposes, additional constructs containing the cauliflower mosaic virus 35S promoter linked to either GUS or *LUC* were also prepared.

These chimeric contructs were then successfully ligated into the polylinker of the binary vector backbone pPZP111 (Hajdukiewicz *et al.*, 1994), for use in plant transformation. A range of these constucts, comprising the *AIM-1* promoter, is shown in Figures 6A(i) to 6A(xii). The backbone vector pPZPIII is shown in Figure 6B. The pGUNt vector is shown

in Figure 6C.

(b) Transformation and regeneration of tobacco

The characterisation of the *AIM-1* promoter was carried out using tobacco as the model plant system. Tobacco transformation was carried out as described by Svab *et al.* (1995). Multiple independent transgenic lines were generated with each of the binary constructs.

(c) Generation of T2 lines

T2 lines were generated from selected T1 lines by self-pollination. Tissue of young transgenic tobacco lines, containing the *AIM-1*:GUS gene construct, were histochemically assayed to visualise GUS activity. Very intense levels of histochemical stain indicate high levels of expression of the GUS gene in tissues of young plants (Figure 7).

(d) Quantitative analysis of *AIM-1* promoter

To quantify levels of expression of the GUS gene under control of the *AIM-1* promoter and compare it to levels obtained using the CaMV35S promoter, quantitative analysis was carried out on two independent transgenic T2 tobacco lines (3-4 and 7-3) containing the *AIM-1:*GUS genetic construct and one transgenic T2 line (5-2) containing the 35S:GUS genetic construct. Assays were performed according to the method of Jefferson (1987) on different plant tissues including root, stem, petiole and first, second and third true leaves. The results indicated that constructs containing the *AIM-1* promoter drive levels of expression 2-5 fold higher than that obtained using the 35S promoter (Figure 8).

EXAMPLE 6

Southern Analysis of Transgenic T2 Tobacco lines

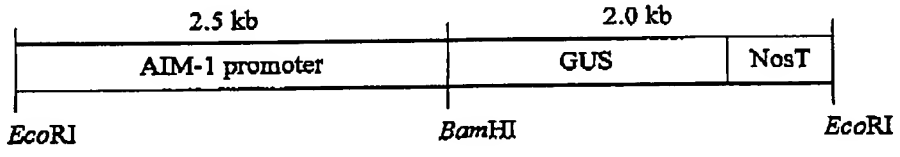
Genomic DNA (10 µg) was digested with *Eco*RI or *Bam*HI restriction enzymes; separated in an electrophoresis gel and transferred to a Hybond™ (Amersham) Nylon membrane. The membrane was prehybridised and hybridised at high stringency following standard procedures (Sambrook *et al.*). A DNA fragment containing the full GUS gene and Nos terminator was labelled with ^{32}P and used as a probe. After washing at high stringency the following results were observed:

a) The *Eco*RI lanes of lines 3-4, 7-3 and 10-3 show a single fragment of the expected 4.5kb size indicating the intactness of the *AIM-1*:GUS:NosT construct in each of the above mentioned lines (see construct diagram below).

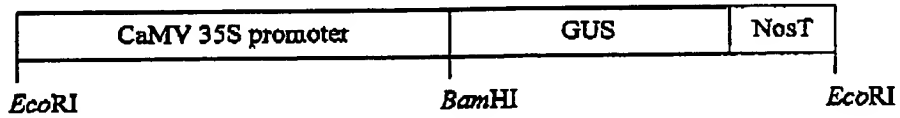
b) The *Bam*HI lanes of lines 3-4, 7-3 and 10-3 show single fragments of different sizes (one fragment per line) indicating the existence of a single copy of GUS:NosT construct in each of the above mentioned lines.

c) The *Bam*HI lane of line 5-2 shows two bands indicating that the line contains two copies of the CaMV 35S:GUS:NosT portion of the construct.

Lines 3-4, 7-3 and 10-3



Line 5-2



These results are shown in Figure 9.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Bibliography

Botella, JR *et al. Plant Mol. Biol. 20*:425-436, 1992.

Botella, JR *et al. Proc. Natl. Acad. Sci. USA 92*:1595-1598, 1995.

Hajdukiewicz *et al. Plant Mol. Biol. 25:*989-994, 1994.

Jefferson, R.A. *Plant. Mol. Biol. Reporter 5(4):* 387-405, 1987.

Sambrook, *et al. Molecular Clonging*: A Laboratory Manual Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, USA, 1989.

Sanger, F *et al. Proc. Natl. Acad. Sci. USA 74:* 5463-5467, 1977.

Selker, JML *et al. Dev. Biol. 153*:29-43, 1992.

Svab *et al.* "Methods in Plant Molecule Biology: A Laboratory Course Manual" pp55-77, Cold Spring Harbor Laboratory Press, NY, 1995.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE UNIVERSITY OF QUEENSLAND

(ii) TITLE OF INVENTION: A NOVEL PLANT PROMOTER AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: AUSTRALIAN PROVISIONAL
(B) FILING DATE: 31-AUG-1998

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: HUGHES, DR E JOHN L
(C) REFERENCE/DOCKET NUMBER: EJH/EK

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +61 3 9254 2777
(B) TELEFAX: +61 3 9254 2770
(C) TELEX: AA 31787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1923 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 88..1542

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATCCTCTCTC CCACTTACTT CGATTTCATC AATTCCAATA AACTCAACAC ACTTTTTTAC      60

ACTCCACACT CTAACCACAT ACACCAT ATG GGT TTC AAG GCC ATG GAC CAA          111
                              Met Gly Phe Lys Ala Met Asp Gln
                                1               5

ACT CCC TTG TTG TCC AAG ATG GCT ATT GGG GAT GGA CAT GGC GAA TCA        159
Thr Pro Leu Leu Ser Lys Met Ala Ile Gly Asp Gly His Gly Glu Ser
     10                  15                  20

TCC CCA TAC TTT GAT GGA TGG AAG GCT TAT GAT CAA AAC CCC TTT CAT        207
Ser Pro Tyr Phe Asp Gly Trp Lys Ala Tyr Asp Gln Asn Pro Phe His
 25                  30                  35                  40

CCC ACA GAT AAT CCT AAC GGT GTT ATG CAA ATG GGT CTT GCT GAG AAT        255
Pro Thr Asp Asn Pro Asn Gly Val Met Gln Met Gly Leu Ala Glu Asn
                 45                  50                  55

CAG CTT ACC TCT GAT TTG GTT GAA GAT TGG ATA CTG AAC AAC CCT GAA        303
Gln Leu Thr Ser Asp Leu Val Glu Asp Trp Ile Leu Asn Asn Pro Glu
             60                  65                  70

GCC TCC ATT TGC ACT CCA GAA GGA ATA AAT GAT TTC AGG GCC ATA GCT        351
Ala Ser Ile Cys Thr Pro Glu Gly Ile Asn Asp Phe Arg Ala Ile Ala
         75                  80                  85

AAC TTT CAG GAT TAT CAT GGT CTG GCC GAG TTC AGA AAT GCT GTG GCT        399
Asn Phe Gln Asp Tyr His Gly Leu Ala Glu Phe Arg Asn Ala Val Ala
     90                  95                  100

AAA TTT ATG GCT AGA ACA AGG GGA AAC AGA ATC ACG TTT GAC CCT GAC        447
Lys Phe Met Ala Arg Thr Arg Gly Asn Arg Ile Thr Phe Asp Pro Asp
105                 110                 115                 120

CGT ATT GTC ATG AGC GGT GGA GCC ACC GGA GCA CAC GAA GTC ACT GCC        495
Arg Ile Val Met Ser Gly Gly Ala Thr Gly Ala His Glu Val Thr Ala
                125                 130                 135

TTT TGT TTG GCA GAT CCC GGC GAG GCA TTC TTA GTG CCC ATT CCC TAT        543
Phe Cys Leu Ala Asp Pro Gly Glu Ala Phe Leu Val Pro Ile Pro Tyr
            140                 145                 150

TAT CCA GGC TTT GAC CGG GAT TTG AGG TGG AGA ACA GGA GTT AAA CTT        591
Tyr Pro Gly Phe Asp Arg Asp Leu Arg Trp Arg Thr Gly Val Lys Leu
        155                 160                 165

GTT CCA GTT ATG TGC GAT AGC TCA AAT AAT TTC GTG TTG ACA AAG GAA        639
Val Pro Val Met Cys Asp Ser Ser Asn Asn Phe Val Leu Thr Lys Glu
    170                 175                 180
```

```
GCA TTG GAA GAT GCC TAT GAG AAA GCA AGA GAG GAT AAC ATC AGA GTA        687
Ala Leu Glu Asp Ala Tyr Glu Lys Ala Arg Glu Asp Asn Ile Arg Val
185                 190                 195                 200

AAG GGT TTA CTG ATC ACC AAT CCA TCA AAT CCA TTA GGC ACA ATC ATG        735
Lys Gly Leu Leu Ile Thr Asn Pro Ser Asn Pro Leu Gly Thr Ile Met
                205                 210                 215

GAC AGA AAG ACA CTG AGA ACC GTG GTG AGC TTC ATC AAT GAG AAG CGT        783
Asp Arg Lys Thr Leu Arg Thr Val Val Ser Phe Ile Asn Glu Lys Arg
            220                 225                 230

ATC CAC CTT GTA TGT GAT GAA ATA TAT GCT GCA ACA GTT TTC AGC CAA        831
Ile His Leu Val Cys Asp Glu Ile Tyr Ala Ala Thr Val Phe Ser Gln
        235                 240                 245

CCC GGT TTC ATA AGC ATA GCT GAG ATA TTA GAG GAT GAA ACA GAC ATA        879
Pro Gly Phe Ile Ser Ile Ala Glu Ile Leu Glu Asp Glu Thr Asp Ile
    250                 255                 260

GAG TGT GAC CGC AAC CTC GTA CAC ATT GTT TAT AGT CTT TCA AAG GAC        927
Glu Cys Asp Arg Asn Leu Val His Ile Val Tyr Ser Leu Ser Lys Asp
265                 270                 275                 280

ATG GGG TTC CCT GGC TTC AGA GTC GGC ATC ATA TAC TCT TAC AAT GAT        975
Met Gly Phe Pro Gly Phe Arg Val Gly Ile Ile Tyr Ser Tyr Asn Asp
                285                 290                 295

GCT GTG GTT AAT TGT GCA CGC AAA ATG TCA AGC TTT GGA TTG GTG TCA       1023
Ala Val Val Asn Cys Ala Arg Lys Met Ser Ser Phe Gly Leu Val Ser
            300                 305                 310

ACA CAG ACT CAG TAT CTT TTA GCA TCG ATG CTA AAT GAT GAT GAG TTT       1071
Thr Gln Thr Gln Tyr Leu Leu Ala Ser Met Leu Asn Asp Asp Glu Phe
        315                 320                 325

GTG GAG AGG TTT CTG GCA GAG AGT GCA AAG AGG TTG GCT CAA AGG TTC       1119
Val Glu Arg Phe Leu Ala Glu Ser Ala Lys Arg Leu Ala Gln Arg Phe
    330                 335                 340

AGG GTT TTC ACT GGG GGG TTG GCC AAA GTT GGC ATA AAG TGC TTG CAA       1167
Arg Val Phe Thr Gly Gly Leu Ala Lys Val Gly Ile Lys Cys Leu Gln
345                 350                 355                 360

AGC AAT GCT GGT CTA TTT GTG TGG ATG GAT TTA AGG CAA CTT CTC AAA       1215
Ser Asn Ala Gly Leu Phe Val Trp Met Asp Leu Arg Gln Leu Leu Lys
                365                 370                 375

AAG CCA ACT TTC GAC TCT GAA ACG GAG CTT TGG AAA GTT ATC ATT CAT       1263
Lys Pro Thr Phe Asp Ser Glu Thr Glu Leu Trp Lys Val Ile Ile His
            380                 385                 390

GAA GTT AAG ATC AAT GTT TCA CCT GGC TAT TCC TTC CAT TGC ACT GAG       1311
Glu Val Lys Ile Asn Val Ser Pro Gly Tyr Ser Phe His Cys Thr Glu
        395                 400                 405

CCA GGG TGG TTT AGG GTG TGC TAT GCC AAC ATG GAT GAT ATG GCT GTG       1359
Pro Gly Trp Phe Arg Val Cys Tyr Ala Asn Met Asp Asp Met Ala Val
    410                 415                 420

CAA ATT GCT TTG CAA CGA ATC CGC AAC TTT GTG CTT CAA AAC AAG GAG       1407
Gln Ile Ala Leu Gln Arg Ile Arg Asn Phe Val Leu Gln Asn Lys Glu
425                 430                 435                 440

GTC GTG GTG TCT AAT AAG AAA CAT TGT TGG CAC AGT AAC TTG AGG CTG       1455
Val Val Val Ser Asn Lys Lys His Cys Trp His Ser Asn Leu Arg Leu
                445                 450                 455
```

```
AGC CTC AAA ACC AGA AGG TTT GAT GAT ATC ACC ATG TCA CCT CAC TCT      1503
Ser Leu Lys Thr Arg Arg Phe Asp Asp Ile Thr Met Ser Pro His Ser
            460                 465                 470

CCC CTA CCT CAG TCA CCT ATG GTT AAA GCC ACA AAT TGAGTTTGCA          1549
Pro Leu Pro Gln Ser Pro Met Val Lys Ala Thr Asn
        475                 480                 485

TATTCCTCTG AATCGTTTAG AAGAAGTAAC TGATATGTGA AGATTACTTG GTTCTTTTAT   1609

TTGTTATTTT GAGAAGGTAC ATAAGTGCTG GATTTGTTCT TTGGAACAGC AATAACAGGA   1669

AATTCCTGAT GTTGTTTTGT GATCGGCATC ACAATCCAGT GTCCTACAAG TTGTGCTGCT   1729

TCATGCACGC CCCTTCAATC TTAGGGGCAT TTTTTCTTTT TTCACTTACC AAAGGTTCAA   1789

GGTGAAAAAA GTTTATAGAG TCTGTAATGT TATTGGTTTA TCAGAAGAGT CCAAAAGATG   1849

TCTGTAATCT GCTACTGAAA TTGTAACTTT CAATTATGAA TAAATTGTTA ATAAAGGTCT   1909

TCAAATTCAT TTCC                                                    1923
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 484 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Gly Phe Lys Ala Met Asp Gln Thr Pro Leu Leu Ser Lys Met Ala
 1               5                  10                  15

Ile Gly Asp Gly His Gly Glu Ser Ser Pro Tyr Phe Asp Gly Trp Lys
            20                  25                  30

Ala Tyr Asp Gln Asn Pro Phe His Pro Thr Asp Asn Pro Asn Gly Val
        35                  40                  45

Met Gln Met Gly Leu Ala Glu Asn Gln Leu Thr Ser Asp Leu Val Glu
    50                  55                  60

Asp Trp Ile Leu Asn Asn Pro Glu Ala Ser Ile Cys Thr Pro Glu Gly
65                  70                  75                  80

Ile Asn Asp Phe Arg Ala Ile Ala Asn Phe Gln Asp Tyr His Gly Leu
                85                  90                  95

Ala Glu Phe Arg Asn Ala Val Ala Lys Phe Met Ala Arg Thr Arg Gly
            100                 105                 110

Asn Arg Ile Thr Phe Asp Pro Asp Arg Ile Val Met Ser Gly Gly Ala
        115                 120                 125

Thr Gly Ala His Glu Val Thr Ala Phe Cys Leu Ala Asp Pro Gly Glu
    130                 135                 140

Ala Phe Leu Val Pro Ile Pro Tyr Tyr Pro Gly Phe Asp Arg Asp Leu
145                 150                 155                 160

Arg Trp Arg Thr Gly Val Lys Leu Val Pro Val Met Cys Asp Ser Ser
                165                 170                 175
```

```
Asn Asn Phe Val Leu Thr Lys Glu Ala Leu Glu Asp Ala Tyr Glu Lys
            180                 185                 190

Ala Arg Glu Asp Asn Ile Arg Val Lys Gly Leu Leu Ile Thr Asn Pro
        195                 200                 205

Ser Asn Pro Leu Gly Thr Ile Met Asp Arg Lys Thr Leu Arg Thr Val
    210                 215                 220

Val Ser Phe Ile Asn Glu Lys Arg Ile His Leu Val Cys Asp Glu Ile
225                 230                 235                 240

Tyr Ala Ala Thr Val Phe Ser Gln Pro Gly Phe Ile Ser Ile Ala Glu
                245                 250                 255

Ile Leu Glu Asp Glu Thr Asp Ile Glu Cys Asp Arg Asn Leu Val His
            260                 265                 270

Ile Val Tyr Ser Leu Ser Lys Asp Met Gly Phe Pro Gly Phe Arg Val
        275                 280                 285

Gly Ile Ile Tyr Ser Tyr Asn Asp Ala Val Val Asn Cys Ala Arg Lys
    290                 295                 300

Met Ser Ser Phe Gly Leu Val Ser Thr Gln Thr Gln Tyr Leu Leu Ala
305                 310                 315                 320

Ser Met Leu Asn Asp Asp Glu Phe Val Glu Arg Phe Leu Ala Glu Ser
                325                 330                 335

Ala Lys Arg Leu Ala Gln Arg Phe Arg Val Phe Thr Gly Gly Leu Ala
            340                 345                 350

Lys Val Gly Ile Lys Cys Leu Gln Ser Asn Ala Gly Leu Phe Val Trp
        355                 360                 365

Met Asp Leu Arg Gln Leu Leu Lys Lys Pro Thr Phe Asp Ser Glu Thr
    370                 375                 380

Glu Leu Trp Lys Val Ile Ile His Glu Val Lys Ile Asn Val Ser Pro
385                 390                 395                 400

Gly Tyr Ser Phe His Cys Thr Glu Pro Gly Trp Phe Arg Val Cys Tyr
                405                 410                 415

Ala Asn Met Asp Asp Met Ala Val Gln Ile Ala Leu Gln Arg Ile Arg
            420                 425                 430

Asn Phe Val Leu Gln Asn Lys Glu Val Val Val Ser Asn Lys Lys His
        435                 440                 445

Cys Trp His Ser Asn Leu Arg Leu Ser Leu Lys Thr Arg Arg Phe Asp
    450                 455                 460

Asp Ile Thr Met Ser Pro His Ser Pro Leu Pro Gln Ser Pro Met Val
465                 470                 475                 480

Lys Ala Thr Asn
```

2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2474 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTACAGATAC ACAGAATCAG ACGACACATC TACTTTAATA ACAGAAAAAT AATAAGTGTC 60

GGAGATTATG GTACGACAAG ATGAAATGTT TTTATATGGT TGAGATTATT TTGGTCTGTT 120

GTTGGAAGTT TCACGAATCA TGATTTTGAT TTTACGTATT AAAAAATGAA AAGTTGAATC 180

ATGCATTTTA TCTAGAAGCT GGGAACTGAA CCAAAAAAAT AGCCAGTTGA ACAACTGCAG 240

TATTTGTAGG CGTATTCATT TCTCCTTTCC TACAATAATC CTTGGTTGCT CTTTATCGGA 300

AAAAAACCAA AAGCAATAGC TACTCTGTAA GGTCCTCGAT TGCCGACAAG AACATCACAT 360

GCGTGCTGTC GAAGAACACA TAATTTTGAG GTTGAAGCTC ACGTGCGAGT TTTGCATATT 420

TTTAGGTTAT GTGTACACGT ATGGAGTGAG TTCCGCGTAT ATAGTGTAGG TAGTTGAGTG 480

GCTGAGTAGC GAGTGAATCA GGTAACACTA TCTTTTCAAG CCACCTAATT AAGGGATTTA 540

ATGTTCATGC AACTGTTCTT CGCTAACTAA GGCCCCACTT ACCTTTATAA TATTCTCTCT 600

AACTCCGGGC TTTTGGTAAG TACAACTTTT CTACTCTTAT TTAATGGAGG GATTATTTTT 660

TCCATATACC AATTAATTTA TTTTTTAATT TATGCATTTT GATCTTATAT TAAAACAATT 720

ATGGTATGGA TTAAGTCGTA TATCGGTGAC AATTGAAGTT TTCCTCAAGT TTAGCCATTT 780

TTATGAAATT AAACTTAATC ACTACTATTA GGTAAATTCA TATGTATCAT TAACAATTTC 840

AATGTGAGTT CAATTTTACC CAAGATTTGA AAGTTGTTGT CAACTTCTGT TAACTAAAGT 900

TGTATTATAA GGTTGACGAC TTTAACCTAA ATCTATTTTG AATTGAAGGG GTTGATGACT 960

TCAGCTTTAA AATAATTCAA CTAAAGTTCT AGACTACATT GGAGATTTTA GTGTTCATAA 1020

AATTTTAGAA AAAGGCTGAG TTAAAGTTAT GAAAAAGATT GGTGACTATT CAATTAATTA 1080

GTTGTGAATT GATGACAAAT ATTTCATGAG CATAACCAAT CAGAGAAATA CCACCTCGAC 1140

CGACTACAAC AATCTCAATG TTAATTAATG AAGCATTGTA GTATAAGGAG TCTAGAATAA 1200

ATTTCTTAAA TATTAGAGGA AAACTATTTT TAAAAAATTA CAAGAAAAGT TTGATCTATA 1260

ACCTCTTTAA ACTTTAAATT ATCTAACAAT TTTCTTATGA CTCACATTGT GTTGATAGGG 1320

TGATTTTGTC AAAATATATG TCTATTTTAT ACTAGTATGA TTTGTCTGCG AATTATATAT 1380

AGTATTAACT TGGAGAAATG ATTGCCTAAT AAGTTATAAA AAAGGAGAAA ATATTTATTC 1440

ATAAAAAAAA TACACTTAAA TAAGTAACAA TAATAAAAAA CATTATATAA GAGATTAAGA 1500

TAATTTAATA AGTATTGAAT GTAGAATAAT TTTTATTTAT AAATTTGAAC TAAAATATTC 1560

AAATAATATT CAAAGTAAAT AATAGATATA ATTCATCATT CAATACGAGT AATTCAATCT 1620

ATTATAATCA TATATTAGAT AAATATACAA ATATTTGTTA AATTTTACAT TATTATATTA 1680

CTAAATATAT ATTAATTTCT TTGAATATCT TTTATACAAG TAGGTAGACT AGAAGAATTA 1740

TCTTATCTCC CGTATATTTG TAGATGTTAA ATGTAACGGG CTTAGACTGA TGTTTTTGTA 1800

TTATATTATT TATAAATCCA TTAGAGATTT AAGTTAATGT CTCTCTTTGA TTTTAACATG 1860

GTTCTAAAAA TTAGGTTTAA TCATTGCGTC CTCAATGAAC CCATGCTATA TGTTTTAAAG 1920

TTTTTTGTTT TTTGACAATG TTTTTTATTT CTGAGATTGC TCTTAGGATT GAAATTATGT 1980

TTGATACTAG AAAACGAAGA AGTAGAGAGT AGTGTATACA CGTGTAAAAA ATAATAGTTG 2040

TGGGAACTTA AGTTGGATTT GAATACTAGG ACGAGGCTGG AAGGGTTTCC ACTAAGTTGA 2100

CAAAAATTAT TACAAGTGGC AACTAGCTAG GTCTCACAAA GTATTACTAA TTAATAGTGG 2160

GTCTGTCTGC ATACCAACTC TTGCCTAATT TTCAAACACC GCATTCTCTC TTCTTCTCTC 2220

CTTCTTCCTC TGGAAACTTC ATCGATGTGG ACTTCTGTCT CTCAAAAGTC AAGCTCAATT 2280

TATCCAATGC ATTATAAATA CACACTCTCC CTCCCTTCTA TTCTTCATTG CATCACATTT 2340

CCTCTATAAA TTACTCACAC CTTATTCCTA ACTTCATTTC AACATCCTCT CTCCCACTTA 2400

CTTCGATTTC ATCAATTCCA ATAAACTCAA CACACTTTTT TACACTCCAC ACTCTAACCA 2460

CATACACCAT ATGG 2474

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGGATCCAT CTTGGACAAC AAGGGAGTT 29

2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TAGGATCCAG AAAGACACTG AGAACCGTGG 30

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ACGGATCCGG TGTATGTGGT TAGAGTGTG 29

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGGATCCAG ACATAGAGTG TGACCGCAA 29

(2) INFORMATION FOR SEQ ID NO:8

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

ATCGATCATA TGAGCTCTAG ACCCGGGCTG CAGGATCCGG TGTATGTGGT TAGAGTGTG 59

(2) INFORMATION FOR SEQ ID NO:9

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

CCGCGGAGAT CTATCGATCT CGAGAATTCA AGCTTCAGAC ATAGAGTGTG ACCGCAA 57

DATED this 31st day of August, 1998

THE UNIVERSITY OF QUEENSLAND

by Its Patent Attorneys

DAVIES COLLISON CAVE

AIM-1 OLIGONUCLEOTIDES

-Oligonucleotide primers used during Long Distance Inverse PCR
-Oligo's bind to regions of AIM-1 (Mungbean ACC Synthase)

NSE-1

5` -GCGGAT↓CCATCTTGGACAACAAGGGAGTT- 3`
29'omer
Tm = 68

NSE-2

5` -TAGGATC↓CAGAAAGACACTGAGAACCGTGG- 3`
30'omer
Tm = 70

NSE-3

5` -ACGGATCC↓GGTGTATGTGGTTAGAGTGTG- 3`
29'omer
Tm = 62

NSE-4

5` -CAGGATC↓CAGACATAGAGTGTGACCGCAA- 3`
29'omer
Tm = 66

NSE-5

5`-ATCGATCATATGAGCTCTAGACCCGGGCTGCAGGATCC↓GGTGTATGTGGTTAGAGTGTG- 3`
59'omer
Tm = 62
note: NSE-5 is identical to NSE-3 except different restriction enzyme sites have been incorporated (ie. 5`-Cla I, Nde I, Sac I, Xba I, Sma I, Pst I & Bam H1-3`)

NSE-6

5`-CCGCGGAGATCTATCGATCTCGAGAATTCAAGCTT↓CAGACATAGAGTGTGACCGCAA-3`
57'omer
Tm = 66
note: NSE-6 is identical to NSE-4 except different restriction enzyme sites have been incorporated (ie. 5`-Sac II, Bgl II, Cla I, Xho I, Eco R1, & Hind III-3`)

FIGURE 1

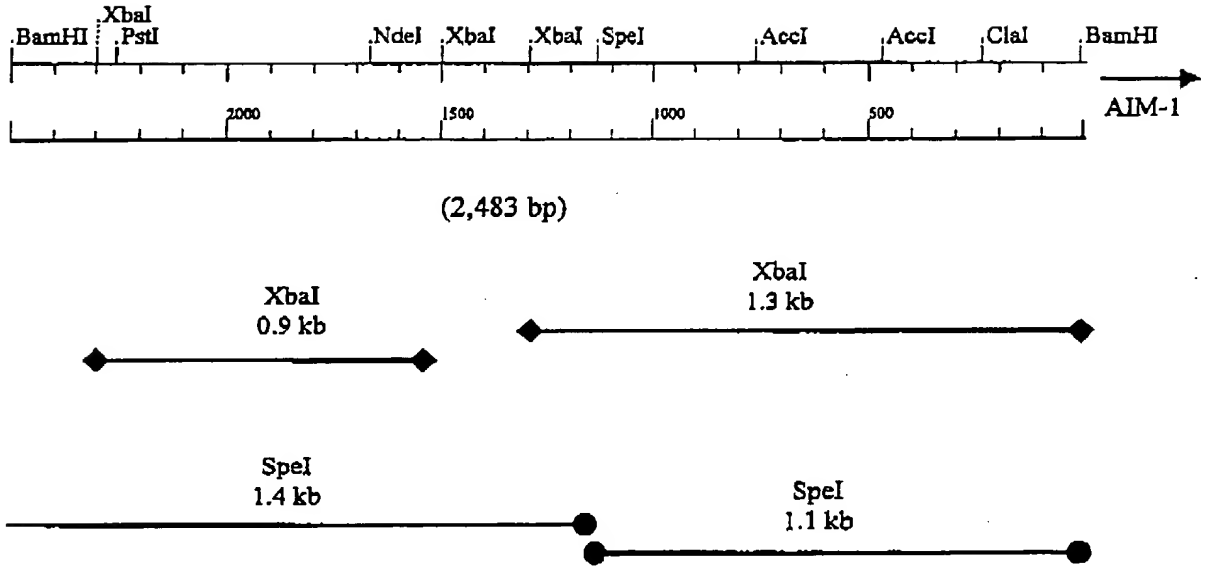

BamHI
XbaI
PstI
NdeI
XbaI
XbaI
SpeI
AccI
AccI
ClaI
BamHI
2000
1500
1000
500
AIM-1
(2,483 bp)
XbaI
0.9 kb
XbaI
1.3 kb
SpeI
1.4 kb
SpeI
1.1 kb

FIGURE 2

AIM-1 2.5Kb promoter sequencing strategy:

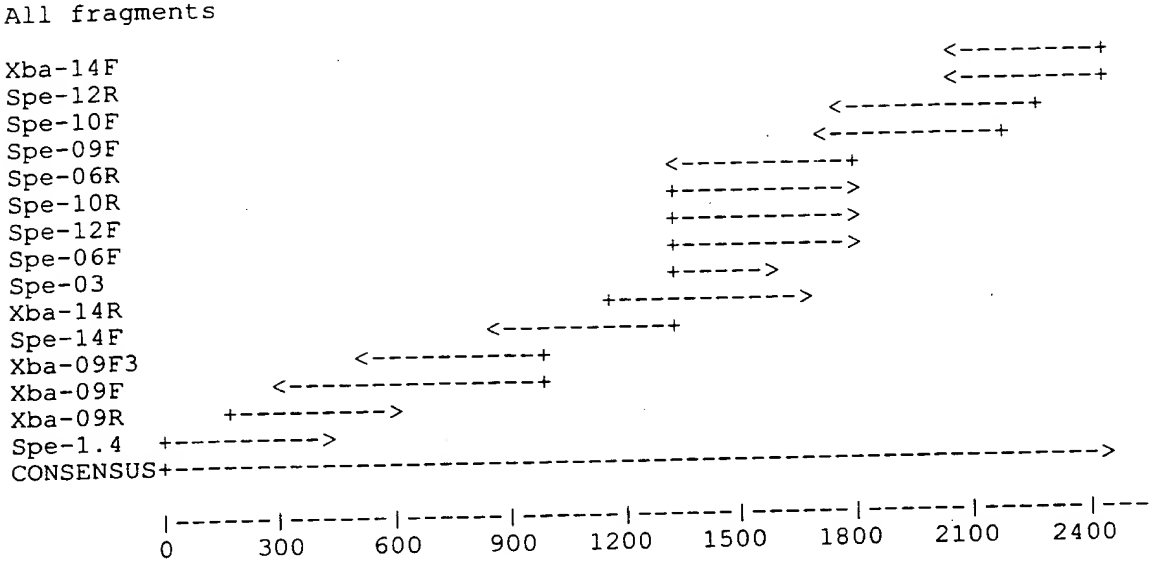


FIGURE 3

IM-1 2.5Kb Promoter Fragment
Length: 2470

```
   1  TTACAGATAC ACAGAATCAG ACGACACATC TACTTTAATA ACAGAAAAAT
  51  AATAAGTGTC GGAGATTATG GTACGACAAG ATGAAATGTT TTTATATGGT
 101  TGAGATTATT TTGGTCTGTT GTTGGAAGTT TCACGAATCA TGATTTTGAT
 151  TTTACGTATT AAAAAATGAA AAGTTGAATC ATGCATTTTA TCTAGAAGCT
 201  GGGAACTGAA CCAAAAAAAT AGCCAGTTGA ACAACTGCAG TATTTGTAGG
 251  CGTATTCATT TCTCCTTTCC TACAATAATC CTTGGTTGCT CTTTATCGGA
 301  AAAAAACCAA AAGCAATAGC TACTCTGTAA GGTCCTCGAT TGCCGACAAG
 351  AACATCACAT GCGTGCTGTC GAAGAACACA TAATTTTGAG GTTGAAGCTC
 401  ACGTGCGAGT TTTGCATATT TTTAGGTTAT GTGTACACGT ATGGAGTGAG
 451  TTCCGCGTAT ATAGTGTAGG TAGTTGAGTG GCTGAGTAGC GAGTGAATCA
 501  GGTAACACTA TCTTTTCAAG CCACCTAATT AAGGGATTTA ATGTTCATGC
 551  AACTGTTCTT CGCTAACTAA GGCCCCACTT ACCTTTATAA TATTCTCTCT
 601  AACTCCGGGC TTTTGGTAAG TACAACTTTT CTACTCTTAT TTAATGGAGG
 651  GATTATTTTT TCCATATACC AATTAATTTA TTTTTTAATT TATGCATTTT
 701  GATCTTATAT TAAAACAATT ATGGTATGGA TTAAGTCGTA TATCGGTGAC
 751  AATTGAAGTT TTCCTCAAGT TTAGCCATTT TTATGAAATT AAACTTAATC
 801  ACTACTATTA GGTAAATTCA TATGTATCAT TAACAATTTC AATGTGAGTT
 851  CAATTTTACC CAAGATTTGA AAGTTGTTGT CAACTTCTGT TAACTAAAGT
 901  TGTATTATAA GGTTGACGAC TTTAACCTAA ATCTATTTTG AATTGAAGGG
 951  GTTGATGACT TCAGCTTTAA AATAATTCAA CTAAAGTTCT AGACTACATT
1001  GGAGATTTTA GTGTTCATAA AATTTTAGAA AAAGGCTGAG TTAAAGTTAT
1051  GAAAAAGATT GGTGACTATT CAATTAATTA GTTGTGAATT GATGACAAAT
1101  ATTTCATGAG CATAACCAAT CAGAGAAATA CCACCTCGAC CGACTACAAC
1151  AATCTCAATG TTAATTAATG AAGCATTGTA GTATAAGGAG TCTAGAATAA
1201  ATTTCTTAAA TATTAGAGGA AAACTATTTT TAAAAAATTA CAAGAAAAGT
1251  TTGATCTATA ACCTCTTTAA ACTTTAAATT ATCTAACAAT TTTCTTATGA
```

FIGURE 4

1301 CTCACATTGT GTTGATAGGG TGATTTTGTC AAAA ATG TCTATTTTAT

1351 ACTAGTATGA TTTGTCTGCG AATTATATAT AGTATTAACT TGGAGAAATG

1401 ATTGCCTAAT AAGTTATAAA AAAGGAGAAA ATATTTATTC ATAAAAAAAA

1451 TACACTTAAA TAAGTAACAA TAATAAAAAA CATTATATAA GAGATTAAGA

1501 TAATTTAATA AGTATTGAAT GTAGAATAAT TTTTATTTAT AAATTTGAAC

1551 TAAAATATTC AAATAATATT CAAAGTAAAT AATAGATATA ATTCATCATT

1601 CAATACGAGT AATTCAATCT ATTATAATCC ATATATTAGA TAAATATACA

1651 AATATTTGTT AAATTTTACA TTATTATATT ACTAAATATA TATTAATTTC

1701 CTTTGAATAT CTTTTATACA AGTAGGTAGA CTAGAAGAAT TATCTTATCT

1751 CCCGTATATT TGTAGATGTT AAATGTAACG GGCTTAGACT GATGTTTTTG

1801 TATTATATTA TTTATAAATC CATTAGAGAT TTAAGTTAAT GTCTCTCTTT

1851 GATTTTAAAC ATGGTCTAAA AATTAGGTTT AATCATTGCG TCCTCAATGA

1901 ACCCATGCTA TATGTTTTAA AGTTTTTTGT TTTTTGACAA TGTTTTTTAT

1951 TTCTGAGATT GCTCTTAGGA TTGAAATTAT GTTTGATACT AGAAAACGAA

2001 GAAGTAGAGA GTAGTGTATA CACGTGTAAA AAATAATAGT TGTGGGAACT

2051 TAAGTTGGAT TTGAATACTA GGACGAGGCT GGAAGGGTTT CCACTAAGTT

2101 GACAAAAATT ATTACAAGTG GCAACTAGCT AGGTCTCACA AAGTATTACT

2151 AATTAATAGT GGGTCTGTCT GCATACCAAC TCTTGCCTAA TTTTCAAACA

2201 CCGCATTCTC TCTTCTTCTC TCCTTCTTCC TCTGGAAACT TCATCGATGT

2251 GGACTTCTGT CTCTCAAAAG TCAAGCTCAA TTTATCCAAT GCATTATAAA

2301 TACACACTCT CCCTCCCTTC TATTCTTCAT TGCATCACAT TTCCTCTATA

2351 AATTACTCAC ACCTTATTCC TAACTTCATT TCAACATCCT CTCTCCCACT

2401 TACTTCGATT TCATCAATTC CAATAAACTC AACACACTTT TTTACACTCC

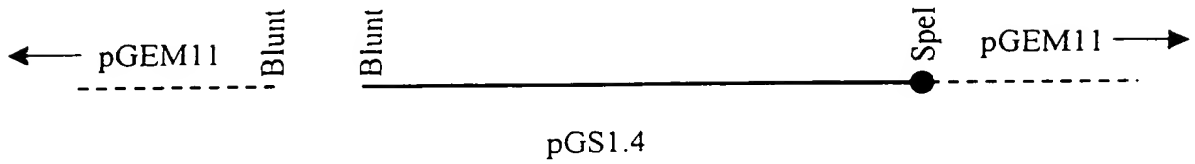
2451 ACACTCTAAC CACATACACC

FIGURE 4 c ntinued

Recor ıction of 2.5 kb AIM-1 pro ter

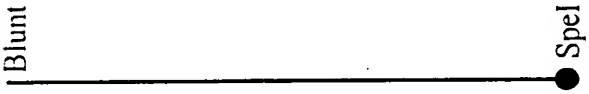
(a)

1. Cut HindIII and blunt end pGS1.4



pGS1.4

2. Cut SpeI



(b)

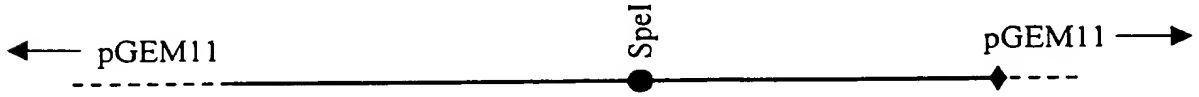
1. Cut SalI and blunt end pGS1.1

pGS1.1

2. Digest with SpeI

pGS1.1

(c) Ligate (a) into (b)



pGS2.5

FIGURE 5

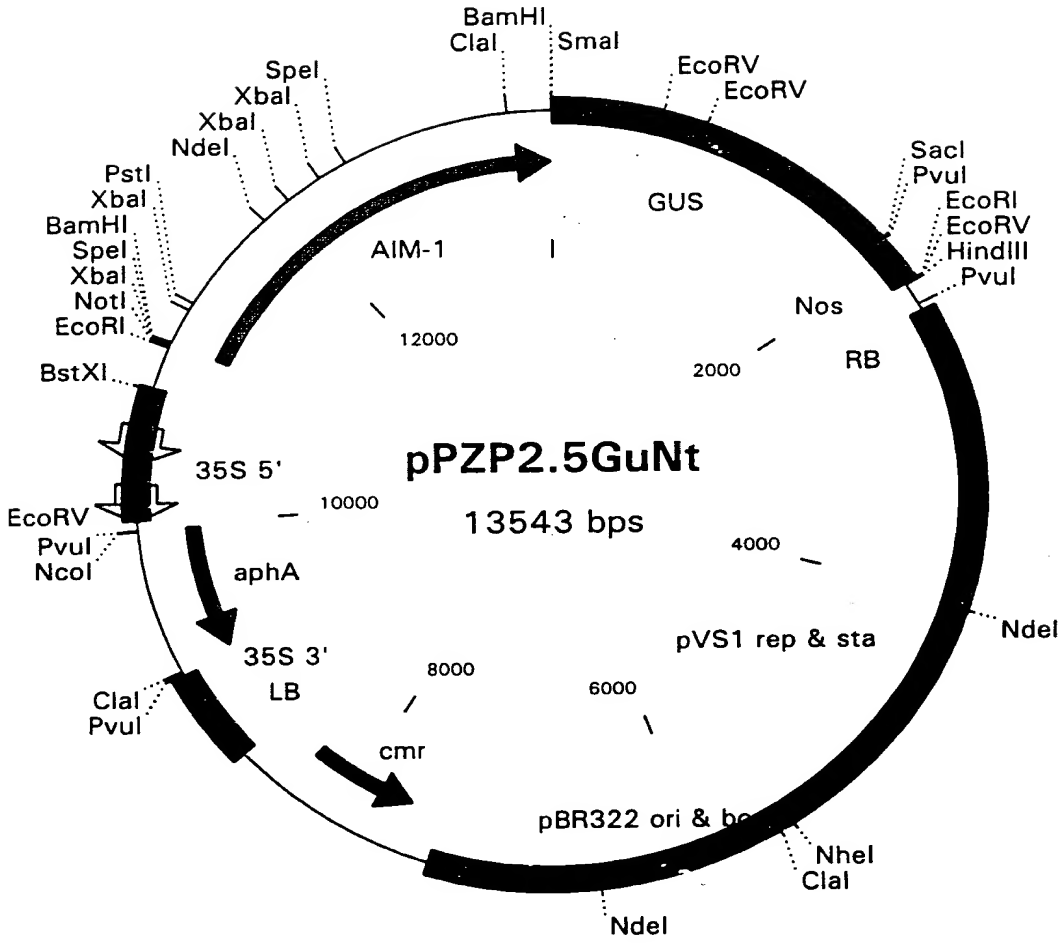

pPZP2.5GuNt
13543 bps
BamHI
ClaI
SmaI
EcoRV
EcoRV
SacI
PvuI
EcoRI
EcoRV
HindIII
PvuI
NdeI
XbaI
XbaI
SpeI
PstI
XbaI
BamHI
SpeI
XbaI
NotI
EcoRI
BstXI
EcoRV
PvuI
NcoI
ClaI
PvuI
NheI
ClaI
NdeI
GUS
AIM-1
Nos
RB
35S 5'
aphA
35S 3'
LB
cmr
pVS1 rep & sta
pBR322 ori & bo
12000
2000
10000
4000
8000
6000

FIGURE 6A(i)

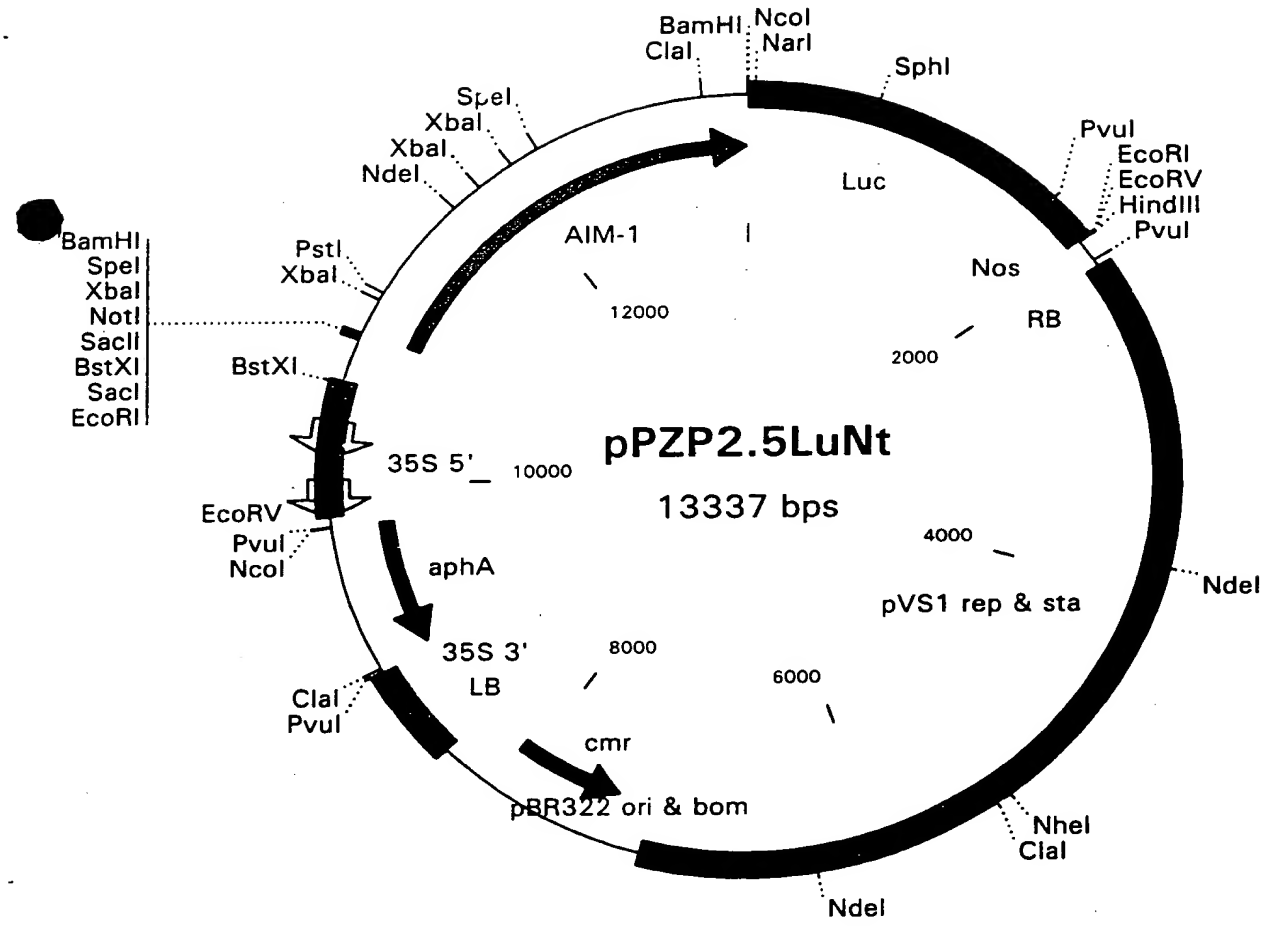

pPZP2.5LuNt
13337 bps
BamHI
ClaI
NcoI
NarI
SphI
PvuI
EcoRI
EcoRV
HindIII
PvuI
SpeI
XbaI
XbaI
NdeI
Luc
AIM-1
PstI
XbaI
BamHI
SpeI
XbaI
NotI
SacII
BstXI
SacI
EcoRI
BstXI
Nos
RB
12000
2000
35S 5'
10000
EcoRV
PvuI
NcoI
aphA
4000
NdeI
pVS1 rep & sta
35S 3'
LB
8000
6000
ClaI
PvuI
cmr
pBR322 ori & bom
NheI
ClaI
NdeI

FIGURE 6A(ii)

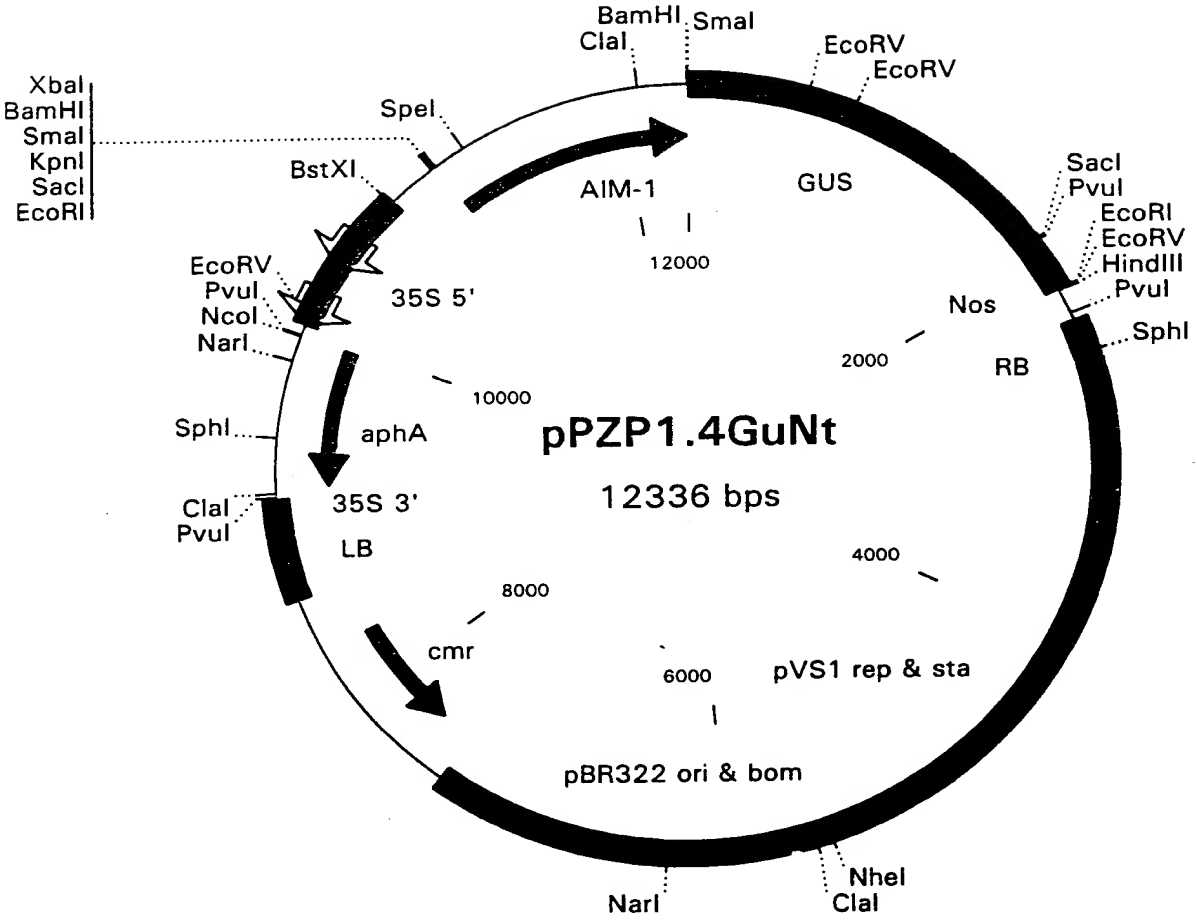


FIGURE 6A(iii)

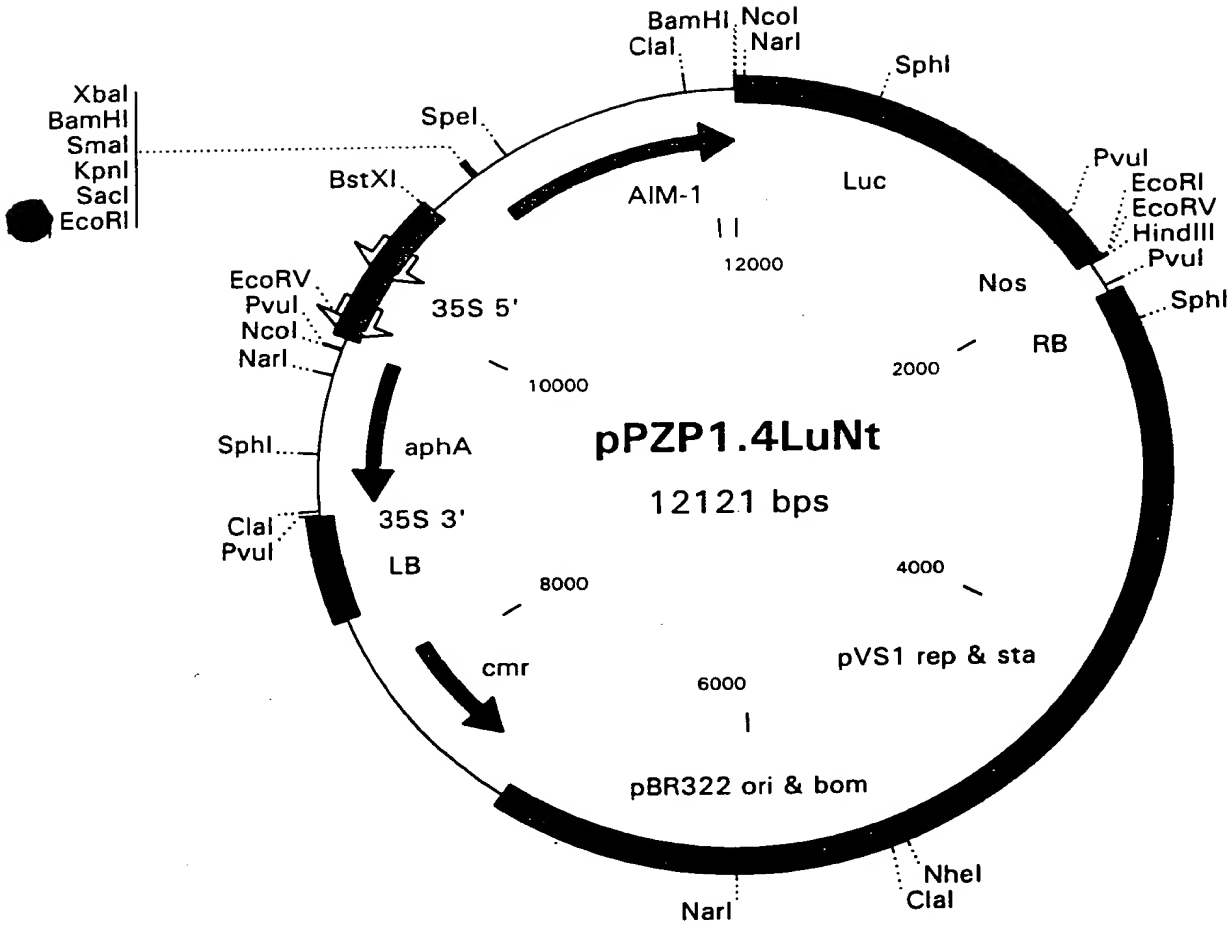

BamHI
NcoI
NarI
ClaI
SphI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI
SpeI
PvuI
EcoRI
EcoRV
HindIII
PvuI
SphI
BstXI
AIM-1
Luc
12000
Nos
RB
2000
EcoRV
PvuI
NcoI
NarI
35S 5'
10000
pPZP1.4LuNt
12121 bps
SphI
aphA
35S 3'
ClaI
PvuI
LB
8000
4000
cmr
pVS1 rep & sta
6000
pBR322 ori & bom
NheI
ClaI
NarI

FIGURE 6A(iv)

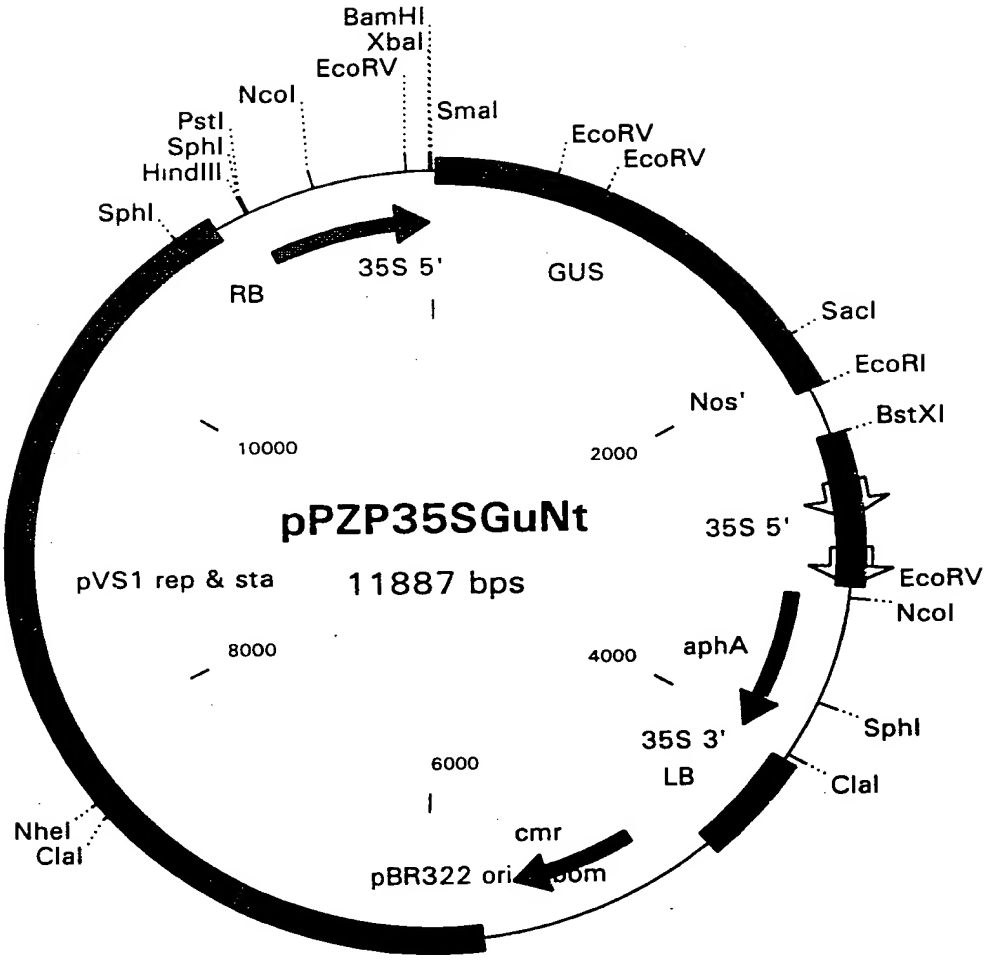

BamHI
XbaI
EcoRV
NcoI
SmaI
PstI
SphI
HindIII
EcoRV
EcoRV
SphI
RB
35S 5'
GUS
SacI
EcoRI
BstXI
Nos'
10000
2000
pPZP35SGuNt
35S 5'
pVS1 rep & sta
11887 bps
EcoRV
NcoI
8000
4000
aphA
SphI
35S 3'
6000
LB
ClaI
cmr
NheI
ClaI
pBR322 ori

FIGURE 6A(v)

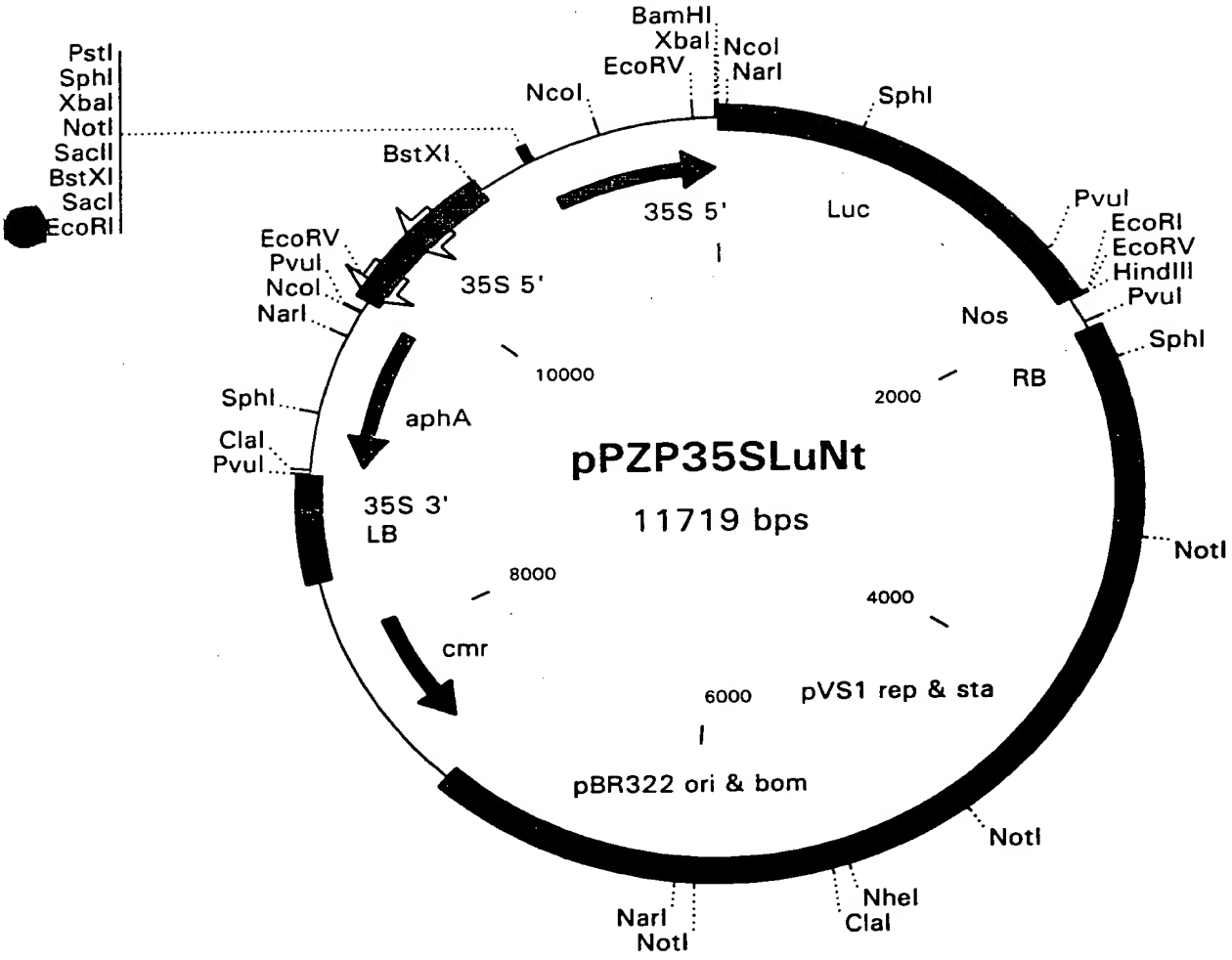

pPZP35SLuNt
11719 bps
BamHI
XbaI
NcoI
NarI
EcoRV
NcoI
SphI
PstI
SphI
XbaI
NotI
SacII
BstXI
SacI
EcoRI
BstXI
35S 5'
Luc
PvuI
EcoRI
EcoRV
HindIII
PvuI
EcoRV
PvuI
NcoI
NarI
35S 5'
Nos
SphI
RB
10000
2000
SphI
aphA
ClaI
PvuI
35S 3'
LB
NotI
8000
4000
cmr
6000
pVS1 rep & sta
pBR322 ori & bom
NotI
NarI
NotI
NheI
ClaI

FIGURE 6A(vi)

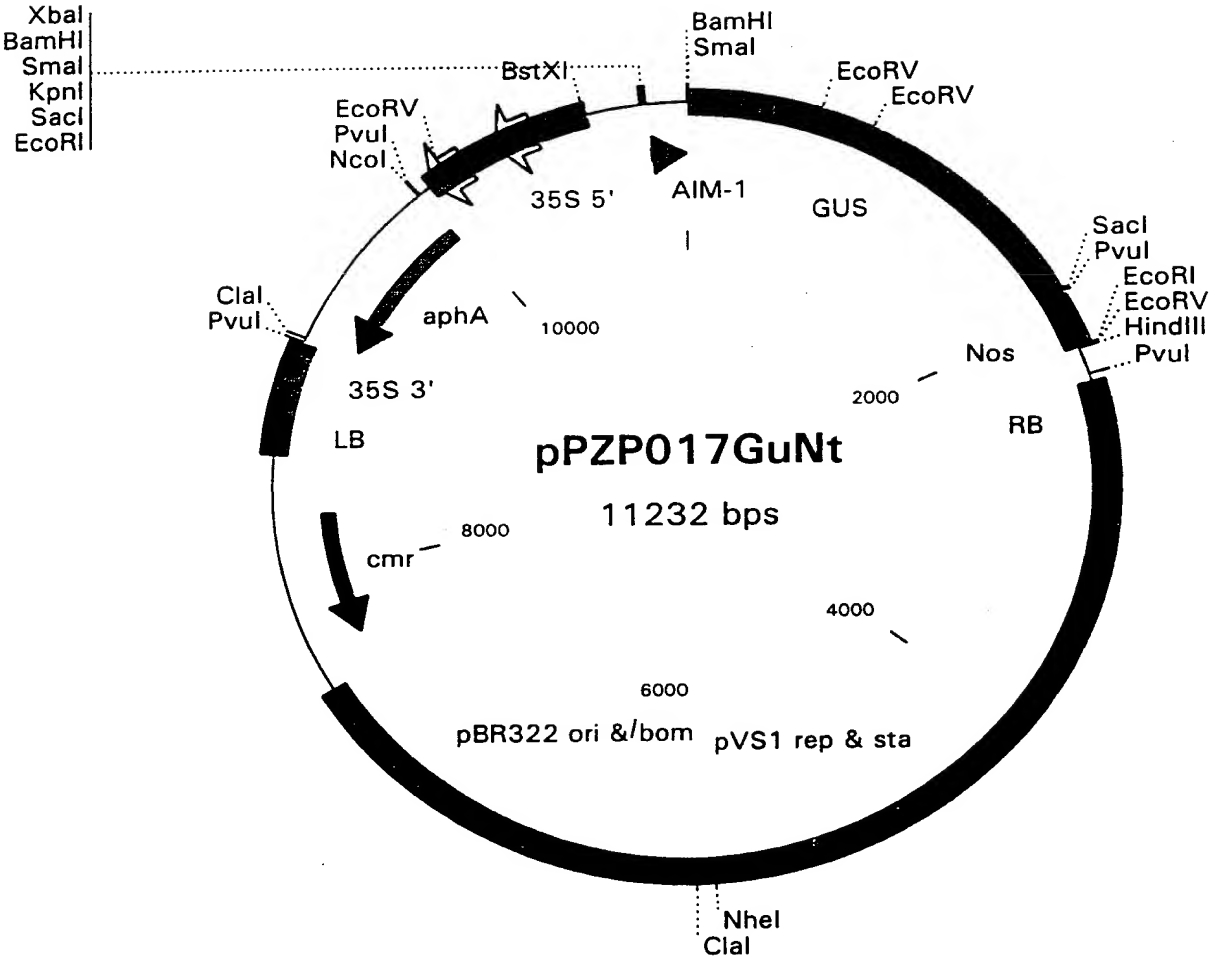


FIGURE 6A(vii)

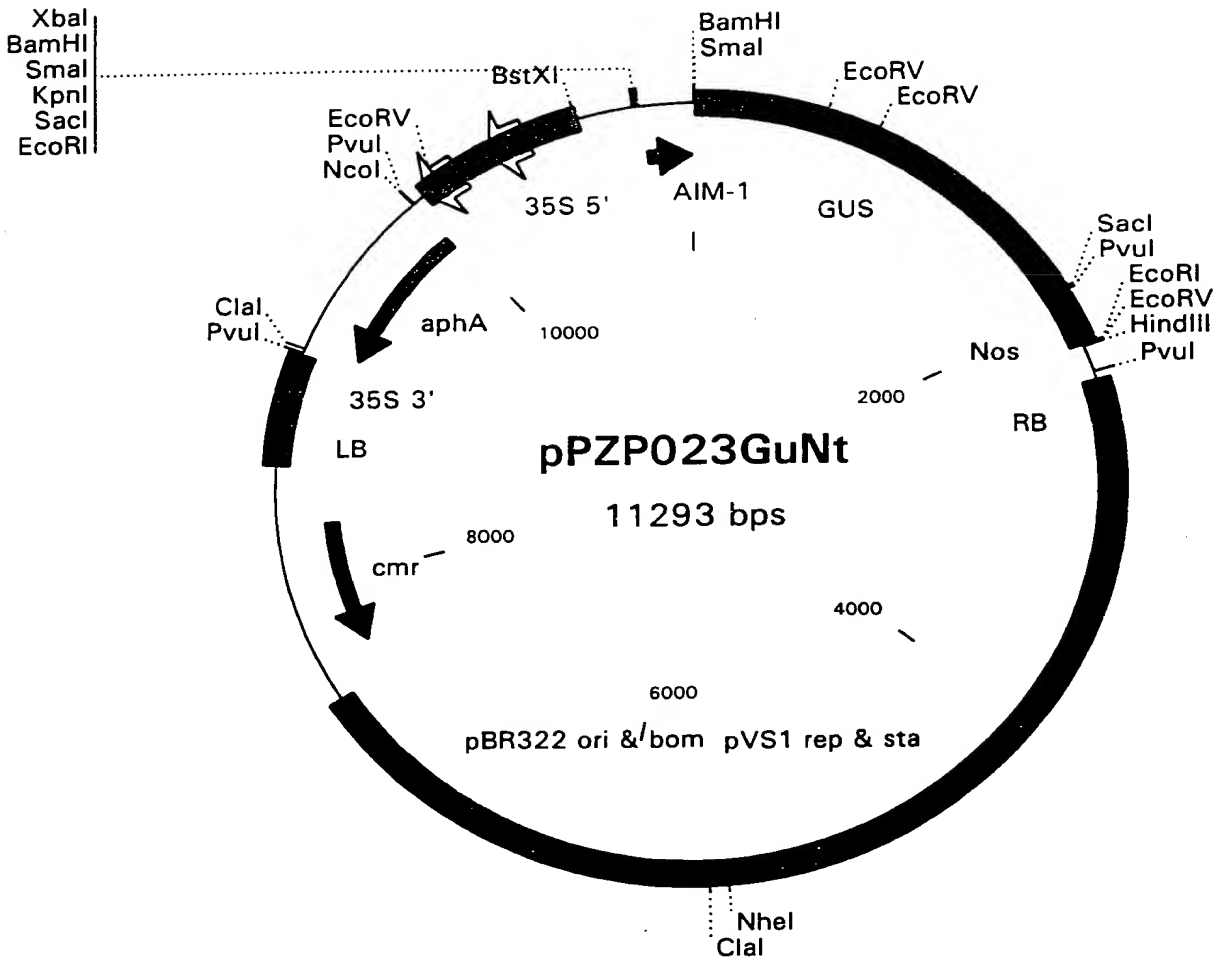


FIGURE 6A(viii)

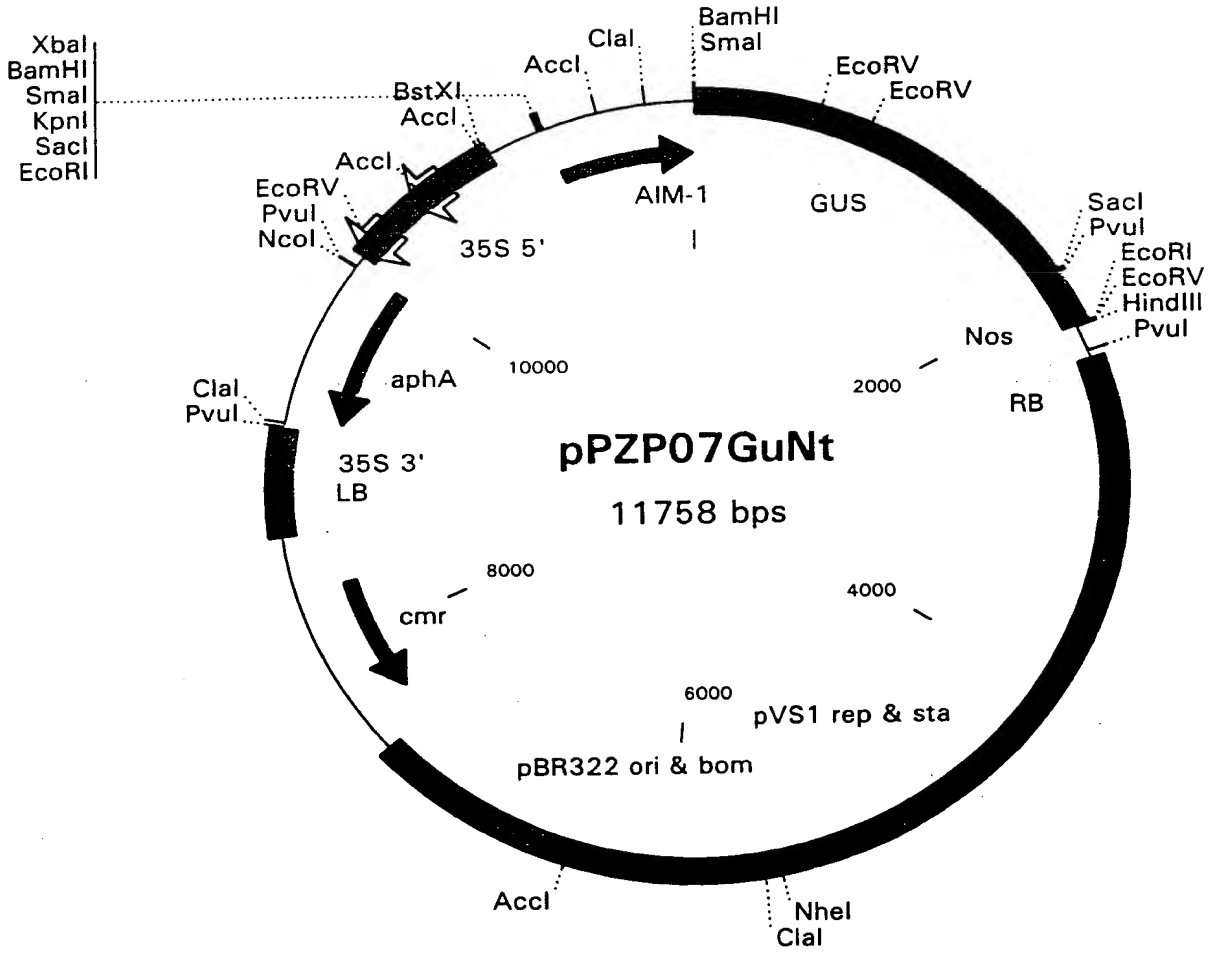

Xbal
BamHI
Smal
Kpnl
Sacl
EcoRI
BstXI
Accl
Accl
EcoRV
Pvul
Ncol
35S 5'
Accl
ClaI
BamHI
Smal
AIM-1
GUS
EcoRV
EcoRV
Sacl
Pvul
EcoRI
EcoRV
HindIII
Pvul
Nos
RB
2000
4000
6000
pVS1 rep & sta
pBR322 ori & bom
Accl
Nhel
ClaI
8000
cmr
ClaI
Pvul
35S 3'
LB
10000
aphA
pPZP07GuNt
11758 bps

FIGURE 6(ix)

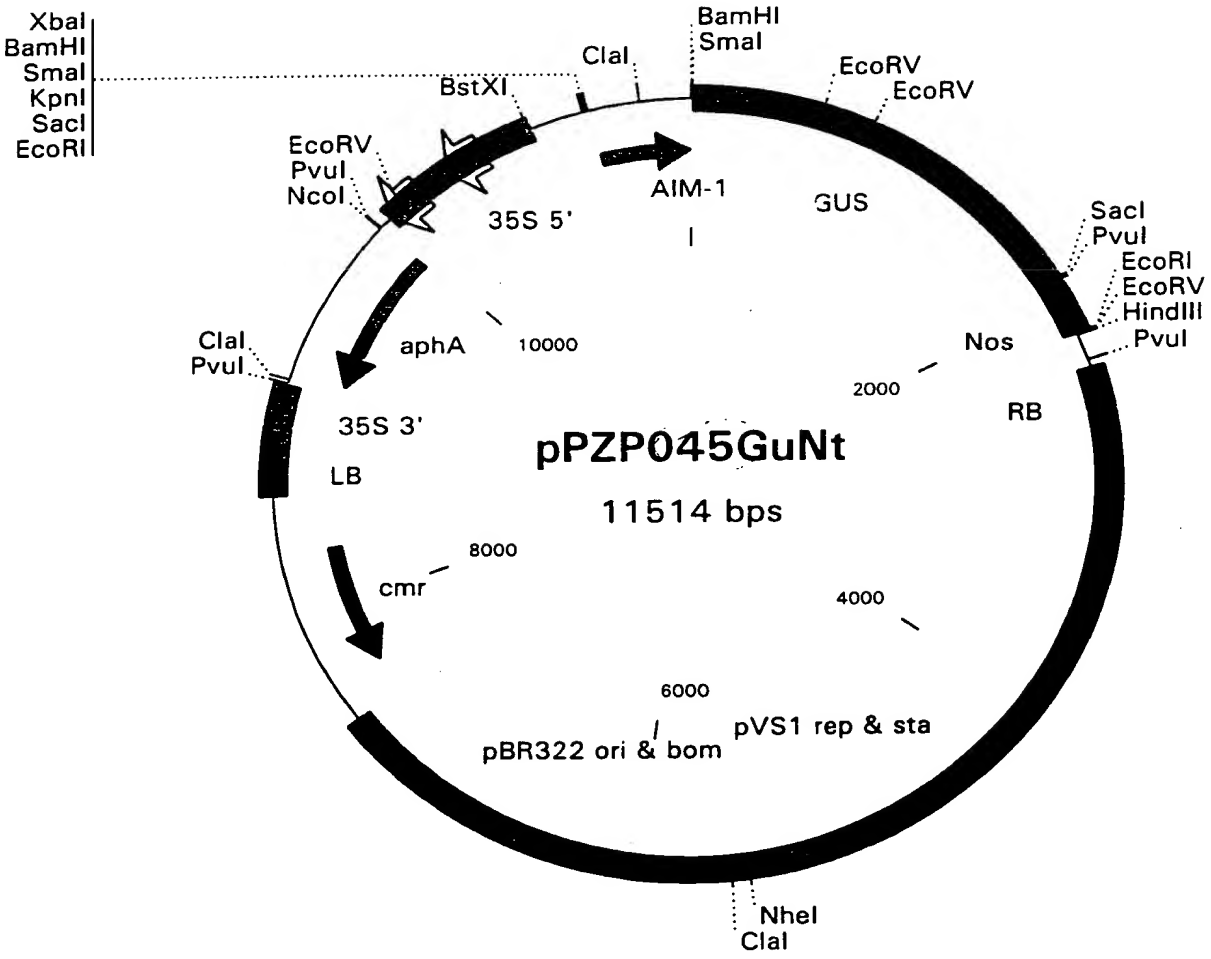

Xbal
BamHI
SmaI
KpnI
SacI
EcoRI
ClaI
BamHI
SmaI
EcoRV
EcoRV
BstXI
EcoRV
PvuI
NcoI
35S 5'
AIM-1
GUS
SacI
PvuI
EcoRI
EcoRV
HindIII
PvuI
ClaI
PvuI
aphA
10000
Nos
2000
RB
35S 3'
LB
pPZP045GuNt
11514 bps
8000
cmr
4000
6000
pBR322 ori & bom
pVS1 rep & sta
NheI
ClaI

FIGURE 6A(x)

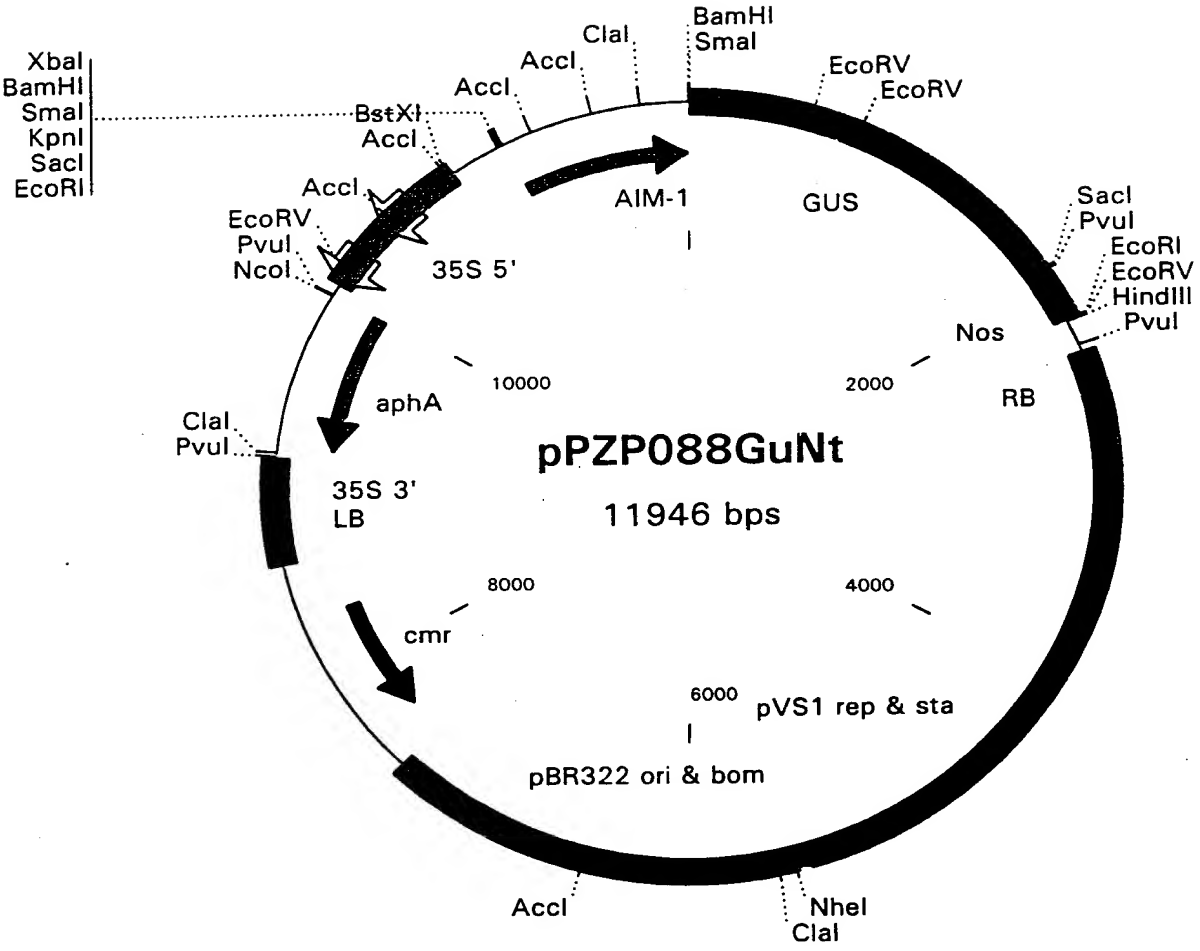

Xbal
BamHI
SmaI
KpnI
SacI
EcoRI
BstXI
AccI
AccI
ClaI
BamHI
SmaI
EcoRV
EcoRV
AccI
EcoRV
PvuI
NcoI
35S 5'
AIM-1
GUS
SacI
PvuI
EcoRI
EcoRV
HindIII
PvuI
Nos
RB
10000
2000
aphA
ClaI
PvuI
pPZP088GuNt
35S 3'
LB
11946 bps
8000
4000
cmr
6000
pVS1 rep & sta
pBR322 ori & bom
AccI
NheI
ClaI

FIGURE 6A(xi)

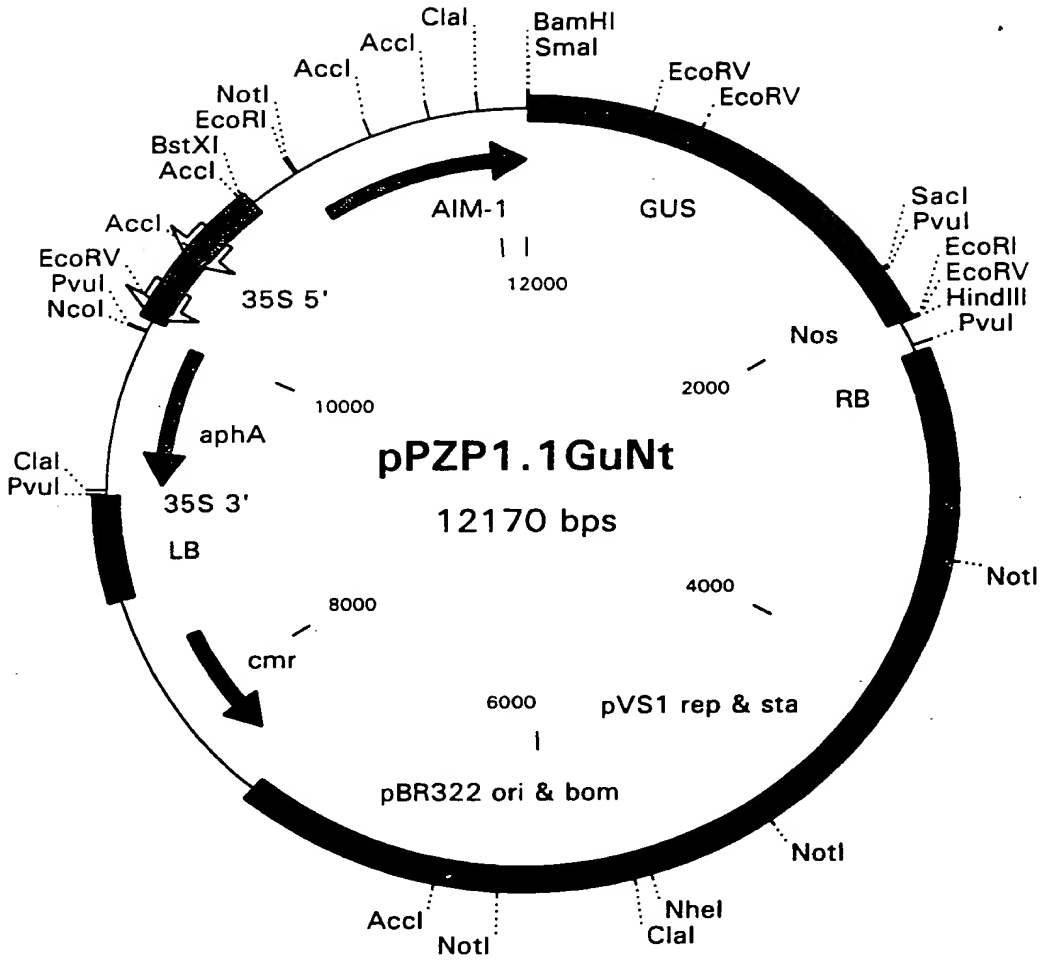


FIGURE 6A(xii)

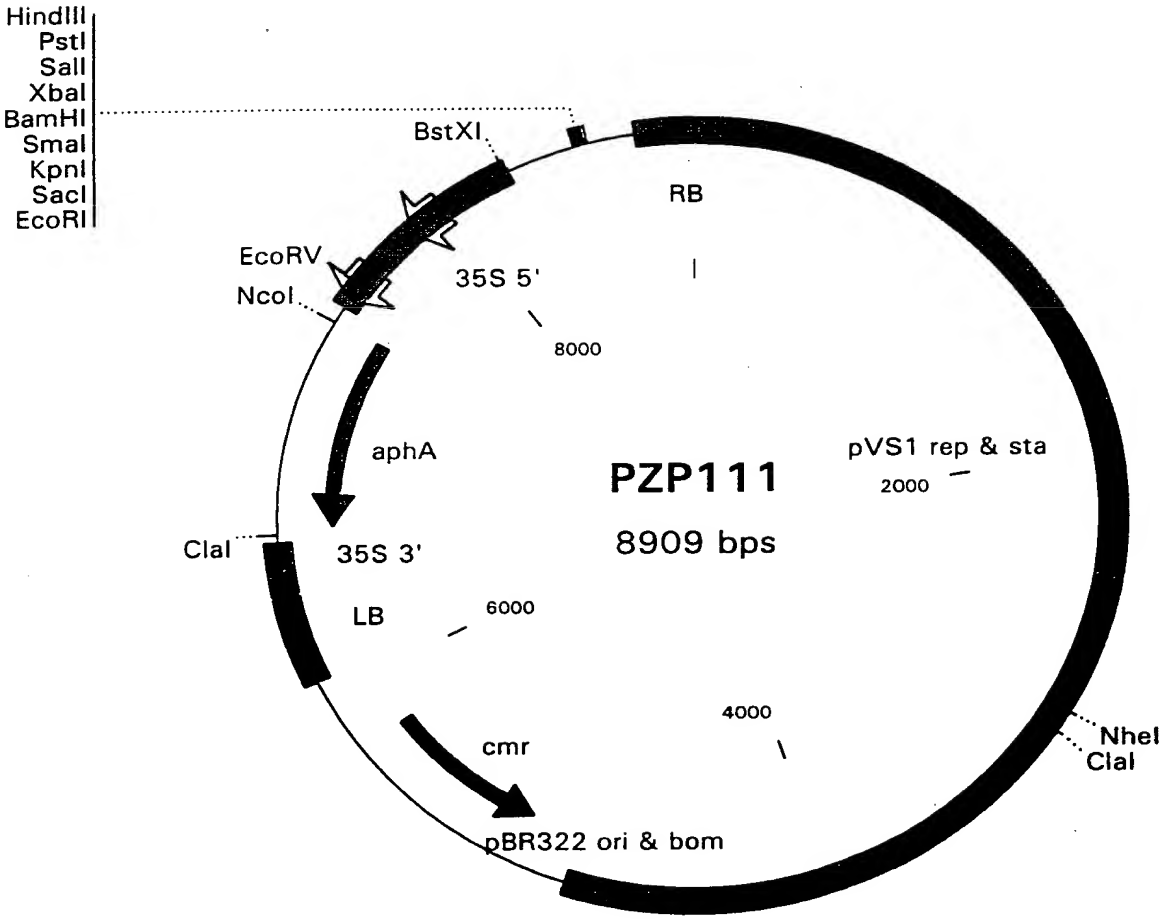

HindIII
PstI
SalI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI
BstXI
RB
EcoRV
NcoI
35S 5'
8000
aphA
PZP111
8909 bps
pVS1 rep & sta
2000
ClaI
35S 3'
LB
6000
4000
cmr
NheI
ClaI
pBR322 ori & bom

FIGURE 6B

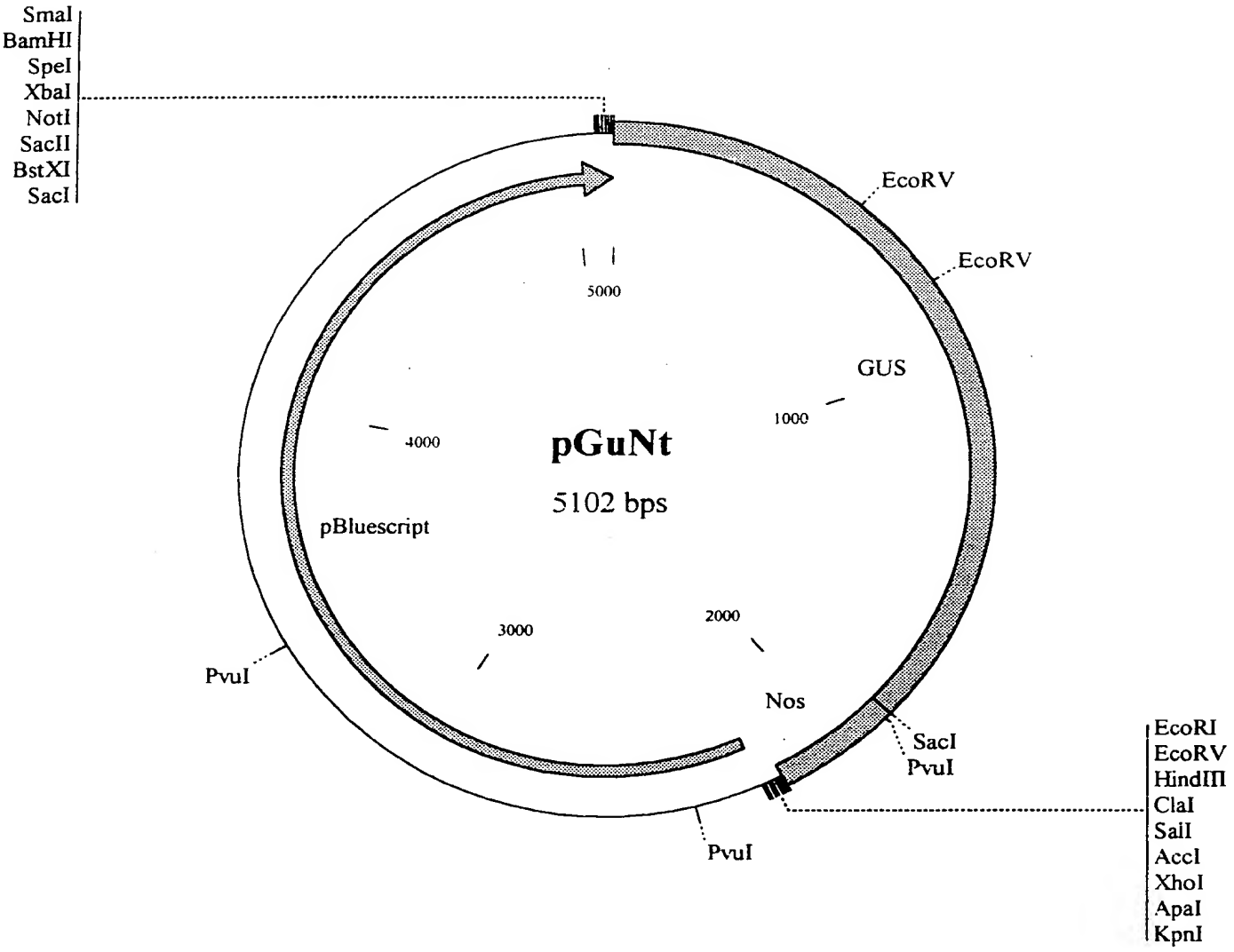

SmaI
BamHI
SpeI
XbaI
NotI
SacII
BstXI
SacI
EcoRV
EcoRV
5000
GUS
1000
4000
pGuNt
5102 bps
pBluescript
2000
3000
PvuI
Nos
SacI
PvuI
PvuI
EcoRI
EcoRV
HindIII
ClaI
SalI
AccI
XhoI
ApaI
KpnI

FIGURE 6C

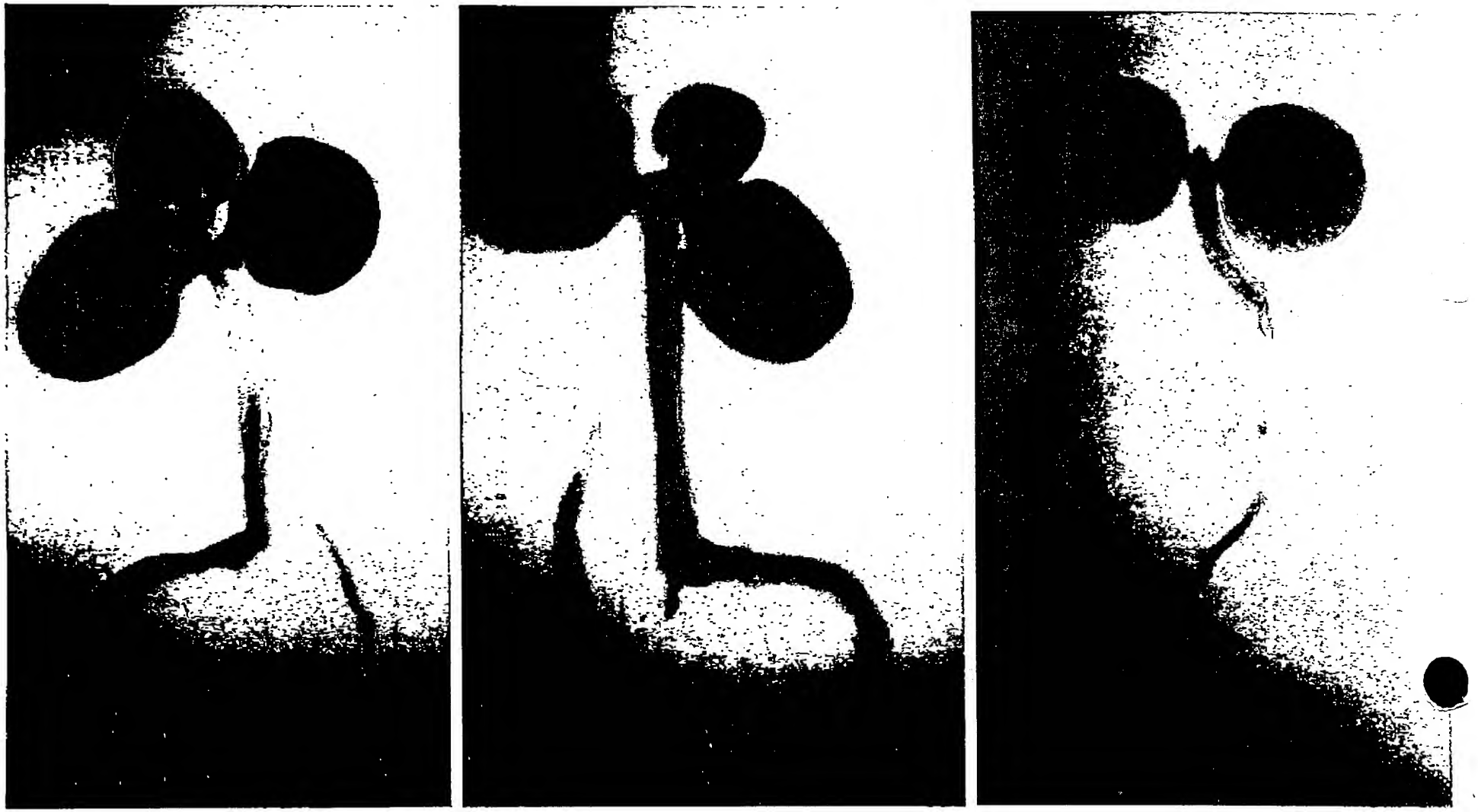


FIGURE 7